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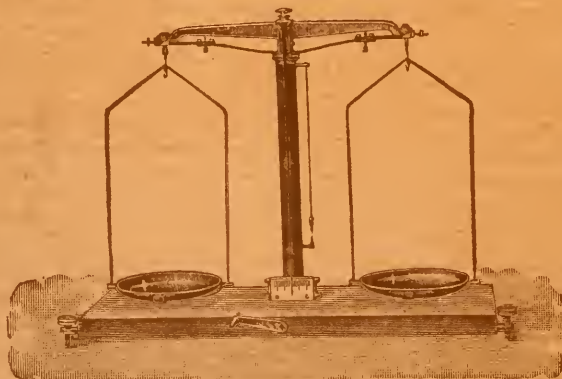
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# Journal of Applied Microscopy and Laboratory Methods

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## Culture Methods with Uredineae.

The cultivation of rust fungi is a process so simple and the results so interesting that it seems strange that it is not more generally practiced. Problems of the greatest importance are capable of solution by this means, and at the same time nothing can be more attractive in class demonstration. In this article some suggestions will be given concerning methods of culture with these fungi.

At the outset it is important to note that these fungi differ from many others of the lower forms of life, particularly the bacteria, in that no particular advantage is gained so far as we yet know in the use of artificial culture media. Many different kinds of culture media have been used with very little effect beyond the results that can be obtained in ordinary water cultures. There is occasionally this difference, that some of the spore forms will produce germ tubes of a little greater length, and growth will proceed a little more rapidly than when simple water cultures are used, but this is about the only difference that has been noticed. All growth stops uniformly at a certain period even in the most carefully sterilized cultures. The writer spent considerable time in an effort to devise a successful artificial medium which would simulate as nearly as possible the conditions obtained in the ordinary wheat leaf. In the case of the common wheat rust, the actual juices of the wheat leaf were used, permeating a solid medium as nearly similar in structure to the leaf as could be made. This, however, failed of its purpose like all other media.

**Methods of Germination.** In ordinary spore germination, the best apparatus for general purposes, that the writer has found, is the ordinary Van Tieghem cell, consisting, as is well known, of a slide with a glass ring affixed to the center within which several drops of water are placed, the material being put in a drop of water in the center of a covered glass which is inverted over this cell. All the different spore forms germinate readily in water, but, as already hinted, in some cases a more rapid germination can be obtained by the use of certain other media—such as solutions of nitrates, beef broth and sugar solutions. Germination goes on best at about the ordinary temperature



of a living room. A little too much heat interferes with the germination just as much as a like decrease in temperature.

In winter the culture should be kept either in a greenhouse or where the temperature can be kept constant by means of a thermostat. In the summer the experiments are usually more successful if the cultures are made in the evening. Under ordinary, favorable conditions the usual amount of growth will be reached in an average time of about 24 hours.

Very little need be said about the use of the microscope in the study of these cultures. A good combination for ordinary study is the No. 6 eyepiece and the 4 mm. objective. This will sometimes be found inconvenient, however, in making drawings with the camera lucida attachment, and in such cases it may be found more convenient to use a 16 mm. objective with an eyepiece of comparatively high power. Of course, a general survey of the culture should first be made with a lower combination, such as a 16 mm. objective with eyepiece No. 4 or No. 6.

**Selection of Material.** There are five well known spore forms of the rust fungi, (1) spermatia, (2) æcidiospores, (3) uredospores, (4) teleutospores, (5) sporidia, the last named being developed directly from the teleutospores. In addition to these, another distinct spore form occurs in the species *Puccinia vexans* Farl. and has been described by the writer and called *amphispore*. This form, however, is extremely rare, but is believed by Dr. J. C. Arthur to occur in at least one other species.

A few hints may well be given here as to the selection of the material among these different forms for germination studies. Until recent years it was not supposed that the spermatia produced regular germ tubes, but that the germination is always simply a process of budding. Dr. N. A. Cobb and the writer have shown, however, that ordinary germ tubes are produced in the germination of these spores as well as in the other spore forms. These observations make it probable that the so-called budding process simply represents instances where the germ tubes have soon ceased their growth in length, and, swelling somewhat in the middle, have given themselves the form of buds. However, it is possible that actual budding occurs. Among the species that have been found by the writer to be particularly good for studying the spermatia, are the rust of blackberry, *Acididium wnothere* Mont.\* on *Onagra biennis*, the ordinary apple leaf rust, and the barberry rust. In the first named species, especially, the spermatia are comparatively large and are readily germinated. Spermatia, though germinating readily in water, will be found to do much better in a rather dilute sugar solution, or perhaps still better in a solution of honey.

The beginner often finds difficulty in germinating æcidiospores which, I think, is usually because the material does not occur in sufficient abundance, so that in collecting the specimens they cannot be kept fresh for so long a time as if there were more material to draw from. Some of

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\*Not to be confused with *Ae. peckii*, De Toni, which has recently been shown by Dr. W. A. Kellerman to be connected with a *Puccinia* on *Carex trichocarpa*, Jour. Myc. 8: 20, May, 1902.

the best species for use in the germination of these spores are the ash rust, the blackberry rust, and the rusts of the verbenas and the euphorbias. The barberry rust is very good, but it cannot often be obtained. Uredospores can, of course, be easily obtained in great abundance all through the summer on the grains and grasses. The best material and most easily obtained, of course, is found ordinarily on the cereals. The three spore forms just mentioned, of course, occur mainly during the summer and are often called summer spores. By wide observation we soon learn, however, that many of these can be very easily obtained during the winter, and the spore cultures can, therefore, be carried on the year round very readily. Uredospores from the grains, and many of the grasses, can be obtained practically every month of the year in almost all latitudes of this country. One needs, however, to become familiar with their habitats and, therefore, be able to trace their existence throughout the winter. In certain species, even æcidiospores can be obtained fresh every month in the winter and germinated. This is particularly true of *Acidum tuberculatum* Ell. and Kell., found in several of the Great Plains states. Spores have been obtained by the writer from this species on plants growing in the snow, and easily germinated.

Teleutospores, with respect to the time and manner of germination, may be divided into two general classes, although they are still further divided in ordinary classification. These two classes are, (1) those that germinate ordinarily at the end of the winter season, and (2) the lepto forms or those which will germinate ordinarily at once after their formation. The rust of velvet leaf (abutilon) is a very good example of a lepto form that is easily obtained in many parts of the country and very easily germinated. Teleutospores of this rust can be germinated, the cultures applied to young plants of the same host, and, after infection, material can be taken from the newly diseased leaves and used in inoculating other plants, and the process repeated over and over again inside of a few weeks' time.

The ordinary stem rust of wheat and oats (*P. graminis*), the black rust of sunflowers and the black rust of wild roses are good examples of those common forms in which teleutospores germinate ordinarily at the end of the season. There are sometimes exceptions, however. The writer has taken teleutospores from the sunflower rust in October which were developed the same season, and produced germination at the time without any period of rest whatever. The germinating spores were even used for infecting sunflower seedlings and the usual spermagonia followed by æcidia were produced. Such facts as these, in connection with known instances where æcidia have been found closely associated with what were supposed to be lepto forms, show that we may occasionally be in error in our classifications. Germination studies, therefore, as will be readily seen, often throw much light upon the problem of classification.

Of the genus *Uromyces*, the species found on Lespedeza, the wild bean, and on certain grasses, especially *Sporobolus*, are very good for a beginning of germination studies with teleutospores. In order to successfully germinate teleutospores after the winter resting period, I probably need not state that the

material should be left outdoors during the winter in order that it may undergo all the ordinary changes of weather that occur in nature.

During the latter part of March or in April, germinations are usually readily obtained with most species. It will be found that germinations will be especially easy if the material has passed through extreme changes of warm, wet weather and freezing temperatures accompanied with snow, such changes following each other rapidly. It is by this means that the writer was finally able to germinate the peculiar amphispores in the species *P. vexans*.

**Methods of Inoculation.** Under the head of Germination Studies I might have mentioned that where it is difficult to keep material fresh on account of having to get it from a distance, the best method is to collect the plants entire, including roots, and keep them in an ordinary tin collecting can. If one wishes to use the material for a week or two weeks, if possible the plants should be taken up by the roots and planted in pots in the greenhouse. By following this method one can soon have quite a collection of plants in the greenhouse with the rust forms from which material may quickly be obtained for carrying out inoculation experiments. In making inoculations, either germinating spores in water cultures may be used, or the material may be taken directly from the infected plant and applied to the healthy plants on which one wishes to make the infection. Because of the additional labor required in making a water culture, it is more convenient to make the infections directly from the infected plant. There will be occasionally instances, however, where, negative results being obtained, one wishes to know whether the spores are really in condition for germination, and, therefore, needs first to make water cultures in order to answer that question. One of the principal objects, of course, in inoculation work is to determine to what hosts the particular rust under study belongs. Aside from this, however, there are many interesting things to the student concerning the manner of the growth of the rust, the period of incubation, etc., which may be learned through these studies. Inoculations for the particular purpose of determining the hosts of a rust are most readily made with uredospores. It is evidently a further confirmation of one's conclusion, however, in the case of heteroecious species if one employs also the other spore forms. Of course, in all inoculations, the new host must be grown from the seed, or one cannot be sure that the rust is not already present.

As most of my own work has been with the grains and grasses, I shall give illustrations from that group of plants. The seed is planted ordinarily in three-inch pots and kept in those pots until the experiments are entirely completed. The inoculations are best made when the plants are from three to five inches above the surface. The manner of making the inoculations is as follows:

Five to a dozen pots, containing each a different host to be inoculated by the same rust, are grouped together on a bench having a bottom of sand, at some point in the greenhouse where there are no great extremes of temperature. Then by means of a thin bladed scalpel, scrape a little material from some of the spots of the infected plants, where it is most abundant,

and apply it at many scattering points over the leaves of each of the healthy plants. These plants should first have been well sprinkled with a good atomizer which produces a very fine spray, thus covering the leaves with very minute drops of water. The rust spores are thus scattered about in these little drops of water. After the inoculations the plants should be again sprayed with the atomizer and then covered with a bell-jar and kept covered for an average of two days' time, after which the bell-jar may be taken off and the plants may remain exposed until the experiment is finished. In the summer season it is best to make the inoculations in the evening. If there is much direct sunshine, the bell-jars should be kept covered with newspapers during the time the sun is shining. In all inoculation work, extremes of temperature and intense sunlight must be avoided. The period of incubation, that is the time from the inoculation until the new rust sori appear, varies considerably for different species, and in the same species will occasionally vary some for different hosts. For the uredo stage of the common leaf rust of wheat (*Puccinia rubigo-vera*) it averages about nine days. For the uredo stage of corn rust this period is considerably shorter, being, in some cases known to the writer, only five days. In other cases the period of incubation is sometimes fifteen to twenty days. This period is always much longer where teleutospores are used to produce æcidia. In such cases the spermatogonia appear first after a week or ten days, the æcidia following a week or more later.

**Suggestive Studies.** I have already mentioned the use of the uredospores in inoculation work with grains and grasses, as being material that is easily handled. In cases of heteroecious rusts, the æcidiospores can be just as readily used for producing other stages on the host to which they belong. For example, if barberry rust happens to exist in sufficient quantity to be readily obtained, material of this rust can just as easily be used to produce other stages of *P. graminis* on wheat and oats as to use the ordinary uredospores. The reverse operation of using the teleutospores of wheat and oat rust to infect the barberry requires a little more patience at first, but can also be readily performed after one becomes a little more skillful in this kind of work. A good species also for the work of beginners is, of course, the common cedar apple which produces the apple leaf rust. In April, after a period of rains, this rust of the cedar germinates so easily that the operation can often be carried out very simply and quickly in watch-glass cultures. The germinating material can then be readily sprayed on the leaves of the apple seedling by means of an atomizer. It seems usually better, however, to simply spray the leaves with water, as already mentioned, and apply the germinating spores independently.

It is with the enormous number of rust forms infesting the grasses that the investigator has the largest field for original work in this line. All the knowledge possible to us from a morphological standpoint alone only leaves these forms in the utmost confusion, and it will require diligent work of a number of specialists for years to place them where they belong.

Inoculation experiments are not only intensely interesting, but are certainly the very best means to be used by the teacher in demonstrating some of the



more common instances of the alternation of stages in the rust fungi, as for examples, the cases just mentioned of the wheat rust and apple leaf rust. Such demonstrations are so clear and convincing that they should be employed in all colleges and academies, without exception, in place of the too common word explanations of lectures and text memorizing. The student is thereby forcibly impressed with the truth of the actual existence of these alternations, whereas he might always remain a little skeptical if his knowledge should go no farther than the mere reading of the text describing former investigations. The writer knows of teachers of science who until very recently did not believe in the actual existence of the alternation of stages in the rust fungi. Of course, they had never seen an actual demonstration.

MARK ALFRED CARLETON.

U. S. Department of Agriculture.

### A Simple Method of Making Wall Charts.

The descriptions of biological wall charts by Orson Howard of the University of Utah, and F. D. Heald of Parsons College, Iowa, published in the JOURNAL OF APPLIED MICROSCOPY, interested me greatly.

Neither of the methods detailed, however, seem to me equal in simplicity to that followed in work for the Histological and Embryological Laboratories of the Woman's Medical College of Pennsylvania.

At the suggestion of Dr. H. H. Cushing, director of the laboratories, under whose supervision the charts were made, I purchased a quantity of the best glazed, white window-curtaining, which was cut into two-yard lengths. The pieces were hemmed at either end, into the lower hem being slipped a cylindrical wooden roller, and into the upper a flat curtain stick, to give the stiffness necessary for convenience in hanging.

The drawing was done with paraffin pencils of the sort usually seen in black for marking freight and express packages. Investigation proved that these pencils were manufactured in all colors by the Acme Pencil Co., Chester, Pennsylvania, and I was able to select shades reproducing very satisfactorily many of the stains, viz., carmine, hæmatoxylin, eosin, picric acid, methylen blue-black, etc., used in mounting microscopic specimens.

The pencils cost but fifty cents a dozen, are economical in use, and the paraffin does not rub.

A faint line can be readily erased with a knife, and I found it convenient to make such a faint plan and sketch of each drawing, and when satisfied of its correctness, to go over the lines heavily, filling in and shading as required.

The pencils are exceedingly easy to use, and although the very fine drawing which is done with pen and ink cannot be rivalled by this method, the work is quite as fine as can be readily seen across a class-room.

In this way some one hundred and eighty very effective charts were made at very little expense, from plates and illustrations selected by Dr. Cushing from fifteen or twenty different text-books on histology, anatomy and embryology, and the students are thus enabled to have the benefit of well illustrated lectures.



The illustration, from a photograph of two charts as they hang before the blackboard, shows (chart A) the different types of epithelial cells, and (chart B) the third day and fifth day chicks.

The charts are kept rolled, held with elastic bands, and stacked in a case



Chart A.

Chart B.

built for the purpose. Each is numbered on the end of the roller, and on the inside of the cover of the case is tacked a card giving each number with a list of figures represented on the chart, and the name and page of the text-book from which they were taken, thus forming a complete index of the illustrations at hand.

MARTHA TRACY.

Woman's Medical College of Pennsylvania.

## A Simple Device for Carrying Minute Objects Through the Grades of Cedar Oil and Paraffin.

In preparing some pollinia of *Asclepias* for embedding in paraffin considerable trouble was experienced through losing quantities of the material in transferring from one grade of cedar oil and paraffin to another. Finally this transfer was very successfully accomplished by enclosing the pollinia in little bags made by bringing together the four corners of a small square (1.5 in. x 1.5 in.) of cheese cloth, and fastening them by one or two turns of small copper wire. One end of the wire was left about one inch long and hooked at the free end. The little bags could be suspended by the hooks in the bottles of oil and paraffin and easily transferred from one to another. Penetration of the tissues by the paraffin was not interfered with at all by the cloth, so far as could be observed. Material thus treated yielded good sections. When the last step, embedding, was reached, the bags were cut from the wire, opened in the melted paraffin, and the pollinia distributed as desired. By this device, excessive handling, which is so injurious to delicate tissue, was avoided.

New York State Normal College.

C. STUART GAGER.

## A Method for Demonstrating Nematocyst Cells in Hydra.

I. Slides prepared by the following method will be found helpful in demonstrating to a beginning class in Zoölogy the number, position and distribution of the stinging or Nematocyst cells of Hydra and others.

Place a number of living Hydra in a Stender dish with a small amount of water. The dish is placed in a good light and the Hydra allowed to become fully extended. A volume of saturated solution of corrosive sublimate ( $\text{HgCl}_2$ ) in 70 per cent. alcohol equal to volume of water in Stender dish is heated boiling hot and poured over Hydra.

The Hydra are killed by this method with body and tentacles fully extended. Wash in several changes of 70 per cent. alcohol, then pass successively through 50 per cent. alcohol, 35 per cent. alcohol, water, stain in an aqueous solution of methylen blue for 5 minutes.

Formula—

Methylen blue	-	-	,	-	1 gram
Castile soap	-	-	-	-	.5 grams
Water	-	-	-	-	300 c. c.

Pass hurriedly through following grades of alcohol, 35, 50, 70, 85, 90, 100 ; clear in oil of cedar or bergamot and mount in balsam.

The Nematocyst cells are stained a deep blue, all other cells are unstained, exploded Nematocyst cells do not take the stain.

This method has been found to give equally good results with Obelia, Gonionemus and other cœlenterates.

II. To stain living Hydra.

Place Hydra in Stender dish with small amount of water ; with pipette place two or three drops of the methylen blue solution directly on the specimen and allow stain to act for from 5 to 10 minutes. The Hydra may then be placed in a watch glass full of perfectly fresh water and examined under  $\frac{2}{3}$  objective. The Hydra will soon recover, the Nematocyst cells will be stained light blue, while the other cells are the natural color.

III. If a large number of Hydra are desired for class use, the following method will be found more satisfactory :

Place the Hydra desired for use in a small vessel ; add enough of the methylen blue to the water in the vessel to make it sky blue. After about two hours the Nematocyst cells of the Hydra will be stained, the Hydra may then be changed to fresh water and are ready for class use.

E. O. LITTLE.

DePauw University.

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TO REMOVE FIXED STOPPERS.—Take the bottle in the left hand with the forefinger applied to one side of the stopper, then tap the other side of the stopper with some heavy instrument, such as the handle of a pocket-knife, pressing the forefinger against the direction of the tap. Turn the bottle round, gradually tapping until the stopper loosens. Should this device prove of no avail (which is very rarely), hold the neck of the bottle in a spirit flame, and quickly withdraw the stopper as the glass of the neck expands. This is a somewhat risky procedure, but is very effectual if done smartly.

## The Museum.

### II.

To arbitrarily group into classes the methods of imparting architectural value to the museum, is open to criticism, exception and apparent disproof. Embellishment plays an important part in the two first methods as in the last, and both the first and second can be and are combined.

It is evident in looking at designs of museums that the windows form attractive temptations for the architect; they can be built up as recesses (Kensington) with mural pillows, they can be mullioned and transomed and obscured at their summits with Catherine wheels and gothic apertures. It seems desirable to change all this. Windows should be large openings, generally rectangular, with shallow or no set-backs, and provided with as large sashes and single glasses or panes as possible. The ideal museum construction in windows could be advocated, of making them sub-convex, giving the sash a depressed bay-window effect, as in a series, excellently designed, in the Macy department store in New York (Fig. 7).

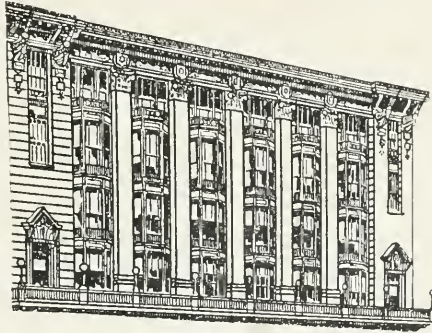


FIG. 7.

The impression left in examining the disposition of windows in museums where the institution reaches imposing proportions is that they were regarded less as entrances for light than ornamental incidents upon which the architect has expended some pains to destroy their original purpose. In this respect the windows of the New York Museum form enviable models. On the exhibition floors the recess from the outer wall scarcely exceeds eighteen inches, they are in double sashes, varied on the third floor by a plain transom of dressed granite. No clustering columns, and no projecting exterior pillars, as in the Provincial Museum, Hanover, obstructs, deflects or baffles the free admission of the light. The vertical walls are broken by semi-cylindrical stone pillars rising to the eaves and surmounted by conventional pediments carrying torches, but these pillars are removed from any proximity to the windows, and make no interference with the latter's natural use. (See Fig. 11.)

The windows in the Brooklyn Institute seem favorable, but there is no conceivable reason for cutting them up into so many minor panes.

Windows, for hygienic reasons, are receiving increased attention in educational and commercial building. Where they best attain their object they run from floor to ceiling, and are broad. Museum windows should follow closely this design, and be relieved of all extraneous interferences. The columns between windows in the Brooklyn Institute apparently are not obstructive (Fig. 8).

Illumination from above by skylights is the second familiar method of securing light in museums. The Field Museum at Chicago, many rooms in the Carnegie Museum at Pittsburgh, and all art galleries illustrate this system. Skylights are in use in the Natural History Museum, London; in the museum of the Royal College of Surgeons, London; in the University Museum, Oxford; Museum of Science and Art, Edinburgh; Public Museum, Liverpool; the



FIG. 8.—Brooklyn Institute, Brooklyn, N. Y.

Science and Art Museum, Dublin; Jardin des Plantes, Paris, and others. It obtains in one hall—the imperfect hall of Conchology—in the American Museum of Natural History, New York, and there yields unsatisfactory results.

In regard to vertical illumination, I think that, excepting in art galleries, the tendency in museum construction should be toward its suppression or abandonment.

To begin with, its effect, owing to the usual circumstance that it is employed in sealed rooms, is lugubrious and church-like. In some instances it is intolerable. It simply drives out the visitor suffocated with a sense of grave-like imprisonment. This applies more exclusively to small rooms. If used at all, let it be combined with lateral lights as well. Vertical illumination on plane surfaces, as pictures on walls, or flat table cases with approximately plane objects, maps, coins, prints, and even shells and minerals, is most serviceable. With



large objects and upright shelved cases, unless supplemented by side lights, it is objectionable.

The device is adopted of allowing a flood of light to descend through a broad skylight for two or more stories illuminating a basement floor, and a series of wall galleries (Natural History Museum, London; Jardin des Plantes, Paris), but it cannot be recommended. The subterranean effect, sometimes the black shadows, the lack of *nuance*, the loss of morning and afternoon lights, make this system extremely unfortunate, uncomfortable and depressing.

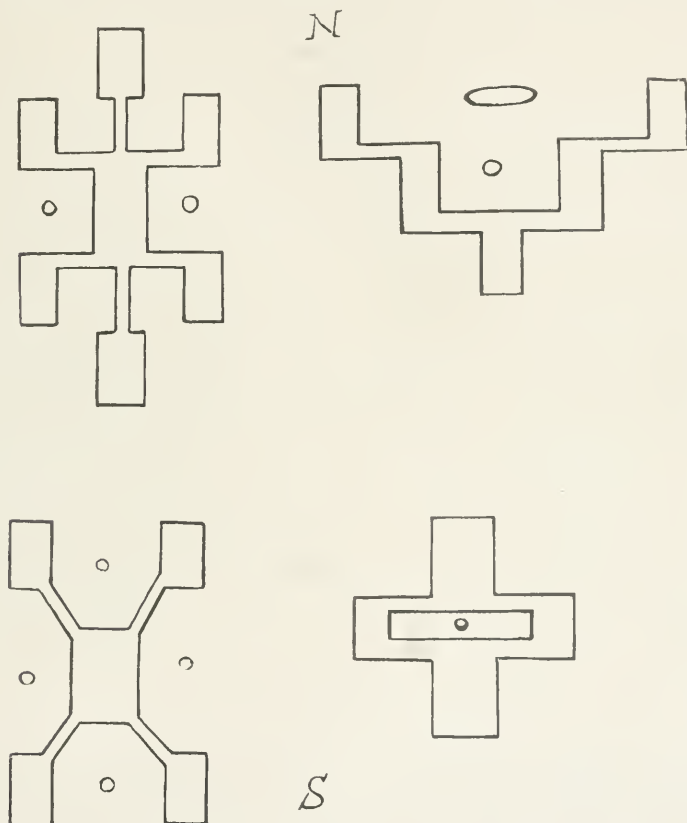


FIG. 9.

A very tantalizing result of total reflexion occurs also with skylights, unless properly obviated, whereby the glass of flat cases, exposed beneath them, becomes the mirrors of the roof, and reflections of gratings or sashes are distractingly mingled with the view of shells and minerals.

The position of the museum building may be considered in this section of the exterior, and remarks made by the author elsewhere (*The Making of a Museum*) retained.

The museum building should be placed in this latitude, and generally north and south, so that morning and afternoon light could enter it. Its width should

be fifty feet and may vary to thirty. Above fifty the illumination is reduced, and below thirty the halls fail to furnish adequate space for economical exhibition. It is impossible to extend one building indefinitely north and south; additions in some way are imperative. Their best disposition, if the ground is available, is in a succession of separated houses arranged so as not to interfere with each other's light and connected by terminal halls.

Groupings (Fig. 9) of this character can be indefinitely varied, and they can be made architecturally attractive separately, and their combination distinctly imposing. But such groupings are usually impossible. They occupy too much ground, they involve an expensive duplication in structure, and they are too scattered, failing in massiveness and solidarity. They besides are more exhaustive of effort and energy to visitors. Yet to such a degree as these long meri-

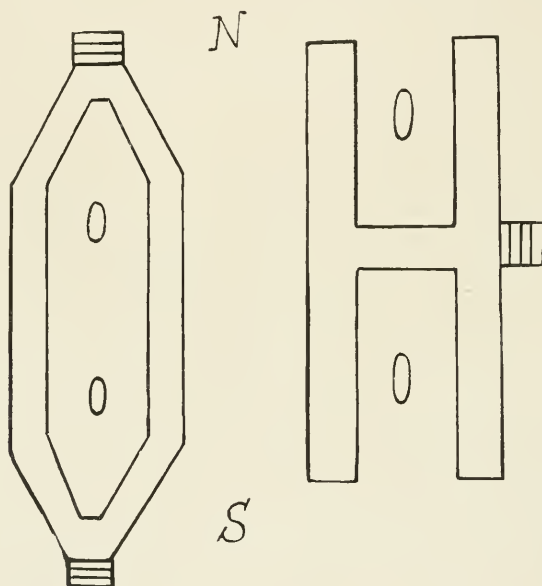


FIG. 10.

dional structures can be obtained in connection with a more reasonable disposition of material they should be desired, because their illumination meets usually the most exacting requirements.

In cogency of design, as involving such an arrangement, a wide elongated court, walled in by the continuous museum buildings with axes north and south, can be recommended. The width of such a court, however, should scarcely be less than five hundred feet, so that the opposite sides of the court should not prove mutually obstructive of light in the mornings and afternoons. The north and south walls connecting the ends of the long side structures will offer a great deal of room, and cannot, of course, be rejected for exhibition uses, but in order to secure light their ceilings should be high and their width greatly narrowed. In this latitude such east and west buildings, if made deep, lose light greatly along the north interior walls. A better plan, as involving less east and west

lines, are two long buildings connected by a narrow hall of one or many stories, which is a corridor of connection and which can be devoted attractively to the illustrative uses of maps, photographs, and pictures (Fig. 10). A still further modification, which provides an almost uninterrupted series of equally lighted halls, is the erection of a prow-shaped terminus to the quadrangle of buildings, formed from two inclined wings meeting in a common entrance (Fig. 10). In this case again the dimensions contemplated are rather greater than is usual, and the complete inspection demands a fatiguing journey, and the conveniences of intercommunication are reduced to a minimum. Still, ideal conditions only are here regarded, and the human factor must retire into extinction.



FIG. 11.—The Museum of Natural History, Manhattan Square, New York, as it will appear when completed.

A museum building can be erected in the form of a rectangle connected by four arms from a central tower, as is the case with the projected complete structure of the American Museum of Natural History (Fig. 11). But the criticism to be made here is the great width—500 feet—of the wings on the south and on the north sides of the rectangle, which are not meant to be connective members simply, but form exhibition halls, which yield defectively illuminated halls on account of their cardinal position east and west. The same length of building north and south would have been preferable. The National Museum at Washington is in the shape of a Greek cross with a central rotunda. The four main arms or “naves” around this rotunda are 101 feet in length and 62 feet wide, and the rotunda rises 108 feet. The exterior angles are filled in with the “courts,” 65 feet square, and these are again flanked by the “ranges,” whose outer walls form the extension of the whole building, which is thus filled out into a complete square. This plan would be most objectionable as far as illu-

mination is concerned, if it were not that it is carried out on a single level with clear-story windows, which contributes also skylight to the general illumination.

Even under the circumstances given, the illumination of the National Museum is not wholly satisfactory, in fact at points is very poor. The limits of time in which there is good lighting are shortened in all single-story roof-lighted buildings, where the walls are very high and the skylight is replaced by clear-story windows, or the skylight has insufficient slope. In regard to the unfavorable condition produced by the combination of high walls and skylights, it has been found that a gradual increasing of the height of the wall greatly diminishes the light. There are in this connection obvious modifications of the shape of a skylight to be considered, according to the latter's length, for architectural effect, viz., a long skylight should not have too steep a slant, and a short one not too low.

The light at this latitude varies significantly in the different seasons, and upon the two opposite aspects (north and south) of a building. In summer the sun reaches at the solstice the extreme northern latitude of  $23\frac{1}{2}^{\circ}$ ; in winter it is never vertical, and the inclined rays in the morning and evening then issue from a point approaching tangentiality with our latitude. The contrast of the north and south sides of a building in illumination is very noticeable from December until April, and hence the meridional position—the flank exposure—is so much to be preferred. To secure the maximum illumination at all seasons, the flat or one storied museum with skylight, clear story, etc., has been devised. It is always best shown in the great exhibition halls of the different World's Fairs, where the possibility of the largest possible public inspection is desired. This result was well attained in the Government Building at the Centennial in Philadelphia (1876), and at the Mines and Mining, Transportation, and Horticultural Buildings at Chicago. In such buildings extreme height must be avoided.

The Manufacturers' and Liberal Arts Building at Chicago was covered, in its skylight, with eleven acres of glass, but its enormous height of 210 feet precluded the full effect of its upper stories and covering of windows. One-storied canopied buildings, if low, are defective in appearance, and they are diffuse and expanded, covering a good deal of ground, while they furnish insufficient isolation, unless cut up into rooms and halls, producing thereby a tangled and confusing labyrinth, interfering with other aims of arrangement.

The museum building can be carried upward to any height, and where space cannot be easily obtained in a north or south line, rather than grow sideways let the museum structure rise upward with additional stories. This has never been tried because it interferes with architectural pretense, but it will keep the museum in the best plane for light as explained above. It is perfectly feasible, and not necessarily ugly. A sixteen or twenty storied bank of halls would, when the very best position had been selected, form an admirable and almost perfect museum structure. The possibilities of ascensional arrangement would permit a very philosophical development of ideas in system and classification, from inorganic through organic to human subjects.



## MATERIAL.

Under the Exterior of the Building, the subject of material is naturally included. Material used in the construction of a museum is determined by financial resources, taste, and convenience. Marble, or marble and brick, gray limestones and brick, brick alone, gray or white granites with brick, red granite alone or with brick, buff or white furnish a range of combinations and effects where the means for building are adequate for such indulgence.

A word of preference may be included. The combination of two materials of contrasted color, if adroitly used, produces more refined effects than a single stone, unless an exception be made in favor of marble.

Generally speaking, there should be but one entrance, unless this may be combined with one exit. As an entrance contains bulletin boards, announcements, and possibly publications, it is desirable to avoid their duplication at a number of points. One entrance also ensures a very correct review of the attendance and its character.

Thickness of walls and all possible safeguards against dampness should be provided for. Discoloration of walls entails expensive replastering, and as repairs in museums are often vexatious and delayed, the best provisions can be made at the outset for their prevention.

American Museum of Natural History.

L. P. GRATACAP.

## A Review of the Methods of Staining Blood.

## IV.

C. *Double Staining with Acid and Basic Stains—Continued.*

4. **Eosin and Methylen Blue.**—Chenzinsky's (1889) eosin and methylen blue solution is as follows: A concentrated watery solution of methylen blue, diluted one-half with water, is mixed with an equal volume of a one-half per cent. solution of eosin in 60 per cent. alcohol. Fixed dry blood preparations are stained four to five minutes in this solution, then washed, dried, and mounted.

*Plehn* (1890) gives the following formula:

Concentrated watery solution of methylen blue,	60
One-half per cent. solution of eosin in	75 per
cent. alcohol, - - - - -	20
Distilled water, - - - - -	40
Twenty per cent. potassic hydrate, - - -	12 drops.

Fix dry preparations in absolute alcohol for from three to five minutes, stain five or six minutes, wash in water, dry, and mount.

*Aldehoff* (1891) stained blood preparations with a concentrated alcoholic solution of eosin [Nr. 22 (bläulich); Fabrik Bayer in Elberfeld] for one-half hour cold, or two to three minutes hot, washed and stained a very short time in a concentrated watery solution of methylen blue, then washed, dried, and mounted.

*Laveran* (1891) recommends the following procedure in successive stain-

ing with eosin and methylen blue: Dry preparations are fixed in alcohol and ether, stained for 30 seconds in a concentrated watery solution of eosin, washed in distilled water and dried. They are then stained for about 30 seconds in a concentrated watery solution of methylen blue, washed again, dried, and mounted.

*Gabritschewsky* (1891) also employed successive staining with eosin and methylen blue for studying the so-called polychromatophile degeneration of red blood corpuscles. Preparations dry heated or fixed in alcohol and ether were stained five minutes in a solution of eosin (1 : 100 parts in 60 per cent. alcohol, diluted one-half with distilled water before using), then washed in water and counter-stained with a concentrated watery solution of methylen blue, which is likewise diluted with an equal volume of water before using, for from one-half to one minute. The preparation is then washed, air-dried, and mounted. Normal erythrocytes stain red, polychromatophile erythrocytes blue red, leucocytes blue, protoplasm red, acidophile granules intense purple, blood plates a mixed eosin and methylen blue color.

*Canon* (1892) employed a mixture in the following proportions for differentiation of acidophile and mast cells:

Concentrated watery solution of methylen blue,	40 grs.
One-half per cent. eosin solution in 70 per	
cent. alcohol, - - - - -	20 "
Distilled water, - - - - -	40 "

Air-dried preparations were fixed at least five minutes in absolute alcohol. The staining was done in a thermostat at 37° C. for from three to six hours.

*Canon* and *Pielicke* (1892) used a mixture with less eosin in order to get a stronger counter stain:

Concentrated watery solution of methylen blue,	- 40
One-quarter per cent. eosin solution in 70 per cent.	
alcohol, - - - - -	20
Distilled water, - - - - -	46

*Mannaberg* (1893) recommends the following procedure: Dry preparations fixed one-half hour in alcohol and ether are dried between filter paper and floated one-half hour upon a half saturated solution of methylen blue, washed in water, dried between blotting paper once more, then stained with a two per cent. solution of eosin in 60 per cent. alcohol for another half hour, washed in water, dried and mounted in xylo-Canada balsam. Both stains may be used combined according to the following formula:

Saturated watery solution of methylen blue,	- - 40
Two per cent. eosin in 60 per cent. alcohol,	- - 80
Water, - - - - -	- - 40

Recently neutral combinations of eosin and methylen blue have become the most important staining reagents in the study of the blood. These will be considered in the section on neutral stains.

5. **Methyl Eosin and Gentian Violet.**—*Hlava* (1883) used this double staining for demonstrating blood plates. Preparations heated above a flame or fixed in concentrated sublimate solution or in alcohol were first stained in a concentrated watery solution of methyl eosin while warm, then shortly in 0.5 per cent. gentian violet. He also stained with fuchsin and methylen blue.

## The Technique of Biological Projection and Anesthesia of Animals.

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### X. THE ANESTHESIA OF ANIMALS—Continued.

**Cœlenterata: *Hydra*.**—Hydras move so slowly that there is little need for anesthesia in studying them alive, but chloretone may be used with fair success in connection with a killing agent in preparing expanded specimens, and with entire success in dissociating the cells while the cells themselves are alive.

To kill hydras expanded, place the animals in a watch-glass with fifty drops of water, or enough to afford them room for full expansion. Allow them to stand quietly until fully expanded. Add one per cent. solution of chloretone carefully one drop at a time at intervals of a few minutes, or as often as the animals are seen to have expanded after the contraction caused by the addition of the drug. When the proportion of chloretone solution equals one-fifth to one-seventh the volume of water in the watch-glass and the hydras do not contract quickly when touched with a needle, add rapidly about two volumes of five per cent. formalin solution or other killing agent, and mount them in hollow-ground slides, or in the usual manner.

To demonstrate the dissociated cells of the hydra, treat as above for killing, but do not add formalin until dissociation is observed to have reached the desired degree and the amœboid motion of the cells has been studied. The gradual separation of the cells may be observed with a quarter or half-inch objective, either in the watch-glass or after transferring the hydra and a few drops of the solution to a slide by means of a pipette. Nematocysts are easily demonstrated, some with threads coiled and others extended. Amœboid motion may also be observed in cells discharged from the hydra's mouth in a brownish colored granular mass when the hydra contracts to its minimum size under the influence of a strong dose of chloretone solution.

**VERMES: *Planarians*.**—These worms are interesting objects for the study of ciliary action and the anatomy of the worm. Place a planarian in a watch-glass, or on a hollow-ground slide in six drops of water and add two drops of one per cent. chloretone solution. Muscular activity ceases in two minutes, or less, but ciliary action may be studied for hours, and is best seen on the sides of the body near the anterior end. The pharynx is protruded in many specimens. When the animals are kept under observation for an hour or more, it is usually necessary to add a drop of chloretone solution occasionally to control returning muscular activity. This is due to two characteristics of chloretone, first, its volatility, and second, the fact that animals become accustomed to it and require larger doses to produce a given effect.

To kill planarians with pharynx protruded, place them in a watch-glass, draw off the water, cover them with one per cent. chloretone solution, and quickly transfer them to five per cent. formalin.

**EARTHWORMS.**—These common animals are of unequaled value for study in

elementary and higher courses in zoölogy, as they exhibit in a remarkably clear manner, when under chloretone, most interesting and important details of structure and functional activities, and a wide variety of tissues. They are readily kept alive in the laboratory throughout the entire year and their manipulation is very easy. Under the influence of the hypnotic, they are perfectly passive in a compressor, and every point in their anatomy may be seen with the compound microscope while studying the functional activity of the circulatory and digestive systems.

For these fascinating studies, the smallest and most transparent worms are to be chosen. Specimens not over two inches long and having a pink tint are best. Wash the worms thoroughly in two or more changes of water, and, if time permits, clear the alimentary canal of most of its dark colored contents by placing the worms, for a day or two, in a deep covered jar with a quantity of wet, clean, white filter paper torn into bits. Lift the animals with a bent needle and place them in a tray with a measured quantity of water sufficient to cover them. Add one-fifth as much one per cent. chloretone solution as there is water. The worms squirm actively for a minute or two, but in three minutes are ready for study with hand lens or compound microscope. The best results are obtained by slightly flattening the worms in a compressor.

A simple and very satisfactory method of mounting them is shown in the accompanying illustration. This gravity-compressor consists of a base plate of ordinary window glass about four by one and a half inches. On this place an ordinary three by one inch slide. On the slide place a few drops of chloretone and water from the tray along with an earthworm. Arrange the worm with dorsal, ventral, or lateral side up, as desired. Across the ends of the slide, place strips of filter paper one fourth inch in width and of enough thicknesses to equal half the thickness of the worm. Moisten the filter paper with the same strength of chloretone solution as used on the worm and drop a little more on the worm, if it is not well surrounded by the liquid. Place another slide over the worm, letting its ends rest upon the strips of filter paper. The weight of its upper slide flattens the worm so that



FIG. 5.—Live Earthworm in Gravity-compressor.

its anatomy is readily seen with any objective having a working distance not less than the combined thickness of the upper slide and the compressed worm. In the illustration a three-fourths inch objective is shown focussed on the "hearts,"



which were pulsating at nearly normal rate during the exposure of two minutes. To study the opposite side of the worm without remounting it, push the two slides to the edge of the base plate, grasp the ends firmly between thumb and finger, turn the slides over with a quick motion so that little or no liquid will run out, and replace them on the base plate. The advantage of the base plate is that the worm is easily moved about under the objective without any irritation of the animal, and at the same time it acts as a tray to catch any liquid from the slides.

Five parts of water to one part one per cent. chloretone solution is the minimum strength, and in it earthworms live for hours and revive when transferred to a quantity of clear water. Occasionally it is necessary to add a drop of one per cent. chloretone solution to the liquid on a slide to quiet an unusually resistant specimen. After using the worms place them in about a half litre of clear water, and in an hour or less transfer them to the jar of filter paper and about ninety per cent. of them will be alive and active and suitable for use the next day.

To kill earthworms expanded, place the washed worms in a tray, cover them with water five parts, one per cent. chloretone solution one part, let them stand for a half hour or more and add enough formalin to make the solution about five per cent. strength.

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## Methods in Plant Physiology.

### VII.

#### CARBON ASSIMILATION—Continued.

8. **Effect of Preventing the Access of Carbon-dioxide to Land Plants.** Plant some seeds of clover (*Trifolium pratense*) or of radish (*Raphanus sativus*) in each of two small crocks of earth which have previously been heated so as to destroy all animal life. When the seedlings have unfolded their primary leaves, each crock is to be placed in a large wide-mouthed bottle, or under a bell-jar with a tubulature at the top. If a bell-jar is used it must stand upon a ground-glass plate, and have the lower edge smeared with vaseline. The method of preparing one of these jars is shown in Fig. 6; a small open dish containing a strong solution of potassium hydroxid is placed inside with the plants for the purpose of absorbing the carbon dioxid in the jar. The jar is closed with a cork, perforated for the passage of a glass tube; all joints are carefully sealed. The air entering the jar is freed from carbon dioxid by its passage through the open U-tube filled with pieces of pumice stone soaked in strong potassium hydroxid interspersed with pieces of solid potassium hydroxid. A control chamber is set up in the same manner, except that the chamber and the ventilating tube have no potassium hydroxid. Place the preparations in strongly diffused (not direct) sunlight; continue the experiment until there is a very noticeable difference in the growth of the two crocks of plants. The plants deprived of carbon dioxid will be found to be deficient in nutrition.

9. **Effect of Preventing the Access of Carbon Dioxid to Water Plants.** The apparatus for this experiment is shown in Fig. 7. The bottle and flasks are fitted with rubber stoppers and glass tubing as indicated.



FIG. 6.

The stopper in A is provided with three holes. The U-tube is filled with pieces of pumice stone soaked in strong potassium hydroxid and a few pieces of solid potassium hydroxid; through it air may enter C. A contains a 10 per cent. solution of potassium hydroxid; B contains water which has been previously boiled and filtered. The clamp between B and C is now closed and the contents of B boiled for fifteen minutes, during which time one perforation in A is opened by removing the glass rod which closed it. As soon as the flame is removed, cautiously insert the glass plug, taking care that none of the solution in A is caused to flow out. B is allowed to stand until cool to the touch.

Place one or two healthy shoots of *Elodea canadensis* or of *Seratophyllum*

*demersum* into C. Open the clamp and siphon water from B into C until the flask is half full. If carefully managed the apparatus will contain so little carbon dioxid that its presence may be neglected for ordinary physiological work. When everything is complete close the clamp tightly enough to exclude all air from C and remove A and B; set C with its U-tube connections in strongly diffused light.

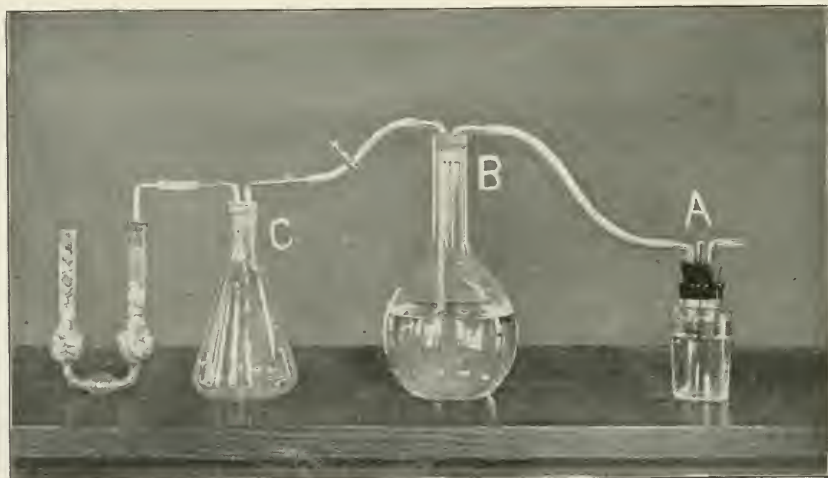


FIG. 7.

A control experiment supplied with carbon dioxide must be set up similar to C, except that the water is not boiled nor passed through B, and the pumice stone in the U-tube is wet with water instead of potassium hydroxide. Each day carbon dioxide should be introduced into the control flask by opening the clamp and forcing one's breath through the tube. At the end of three or four days the plants are to be taken from the flasks, the chlorophyll extracted and tested for starch. The plants will show that starch formation does not proceed in the absence of carbon dioxide.

10. **The Relation of Light to the Evolution of Oxygen.** The well known formula,  $6\text{CO}_2 + 5\text{H}_2\text{O} = \text{C}_6\text{H}_{10}\text{O}_5 + 6\text{O}_2$  shows that six molecules of oxygen are given off as a by product in the formation of a molecule of starch. It is evident then that the rate of evolution of oxygen may be taken as an indication of the amount of carbon assimilation. The evolution of the gas is easily observable in certain water plants. Fill a small, four-sided glass jar with water at a temperature of  $20\text{--}22^\circ\text{C}$ ., during the course of the experiment the temperature of the water must not vary outside of these limits. Select a vigorous stem of *Elodea canadensis* 5 to 8 cm. in length and tie it to a glass rod. Pinch off the proximal end of the stem with the fingers and insert rod and plant in the jar, with the apex of the stem downwards. The jar is to be placed in direct sunlight and the evolution of bubbles observed as they rise through the water. If the bubbles do not come off in single file, or are too rapid to be counted, another bit of stem should be pinched off.

When the experiment is working well, place screens of thin, white tissue paper or ground glass plates between the plant and the window, continue to reduce the amount of light until a further reduction would diminish the number of bubbles. This may be designated as the optimum amount of light. In this light, with the stop-watch in hand, determine the time required to produce a given number of bubbles. Make at least five determinations and average them for the final record.

While the screens are still in position cover the plant with a double-walled bell-jar containing a saturated solution of potassium dichromate and determine the rate of evolution of bubbles. Replace the bell-jar with another containing an ammoniacal copper sulphate solution and determine the evolution of bubbles.

11. **Dependence of the Evolution of Oxygen on Carbon Dioxide.** Pour out the water from the jar used in the last experiment, and fill the jar with water from which the carbon dioxide has been removed as in flask B, Fig. 7. The tube through which the water enters should reach to the bottom of the jar containing the plant, and all agitation of the water should be avoided. The evolution of bubbles in the sunlight should be observed; then the observer may, by means of a tube, force his breath through the water. Notice that the evolution of oxygen is accelerated by the introduction of carbon dioxide.

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## The Bacterial Flora of Freshly Drawn Milk.

## III.

The methods employed in this work were essentially those used by Ford; a large piece of the tissue to be examined was excised with a sterile knife, placed in a sterile jar, and immediately taken from the slaughter house to the laboratory. Small pieces of tissue were then cut from the *inside* of the large piece with sterilized knives, and then held in the flame of a Bunsen burner with sterilized forceps until the whole of the outside of the piece was well scorched. The piece was then transferred to beef bouillon or peptone whey bouillon, and the preparations placed in the incubator at 37°. On the fourth day gelatine plates were made from the different pieces of tissue, and the bacteria, if any, were isolated.

1. An aged cow, dried up five weeks before slaughtering, udder small, with considerable fatty tissue. All organs perfectly healthy and normal.

<i>Liver</i> ,—	Bouillon	Peptone Whey
	+	+

Subsequent plating and sub-cultures gave  
*B. mesentericus vulgaris*.  
*B. subtilis*, and a Micrococcus, identity not established.

<i>Udder</i> ,—	Bouillon	Whey Peptone
	+	+

Plates gave colonies of  
*B. subtilis*.  
 Micrococcus (sp?).

2. An aged cow, dry for some time, the butcher not knowing the exact length of time. Udder of a fair size and well formed. All organs apparently healthy and normal.

<i>Liver</i> ,—	Bouillon	Whey Peptone
	+	+

Plates gave colonies of  
*B. lactis aerogenes*.  
 Proteus.

<i>Udder</i> ,—	Bouillon	Whey Peptone
	—	—

3. An aged cow, dry for four weeks previous to slaughter. Udder fair size. Organs normal and apparently healthy.

<i>Liver</i> ,—	Bouillon	Whey Peptone
	+	+

Gelatine plates gave colonies of

*B. subtilis*.

A spore bearing bacillus, which produces no effect in milk.

<i>Udder</i> ,—	Bouillon	Whey Peptone
	+	+

Gelatine plates gave cultures of

*Micrococcus varians lactis*.



These results, whilst agreeing with Ford's, are not sufficiently authoritative to allow us to assert positively that the bacteria found in the udders of the two cows came from the blood or lymph stream, rather than through the teat, but in conjunction with the results obtained by Ford, they throw doubt on the supposition that all udder infection comes originally through the orifice of the teat. It is also noteworthy that a spore bearing bacillus belonging to the subtilis group, and several micrococci were isolated by Ward from udder tissue. Another fact which is difficult to explain, and which may possibly have some influence on the bacterial content of the normal udder, is the strong germicidal power of freshly drawn milk. This property was first noticed by Fokker (22), and subsequently confirmed by de Freudenreich (23), and quantitative studies of freshly drawn milk inoculated with various bacteria showed that an actual destruction of bacteria took place. This germicidal property has been shown to exist in the milk serum, and is evidently allied to the similar bactericidal property of blood, for Brieger and Ehrlich (24), and Wassermann (25) have found that the milk of immune animals can confer immunity.

If then this germicidal power exists in fresh drawn milk, it is certain to be present whilst the milk is still in the udder, and may inhibit or prevent the rapid multiplication of adventitious bacteria, which penetrate up the opening of the teat. Although we have frequently found large numbers of lactic acid bacteria in freshly drawn milk, yet the reaction of this milk is never acid, but usually amphoteric to litmus.

De Freudenreich has also shown that the bactericidal power is not the same for all species, that whilst the cholera vibrio, the typhoid bacillus, and even *B. Schafferii* (a colon bacillus) are destroyed in large numbers, the bactericidal action is less pronounced on lactic acid bacteria. These facts may possibly explain why the germs of the Colon type are so seldom found in the healthy udder, for we know that the teats and udder of the cow are constantly brought in actual contact with particles of manure, and even the hands of the average milker are soiled with stable filth, which undoubtedly contains Colon bacteria.

It might be reasonably asked if the advice, commonly given to those who wish to procure milk as near sterile as possible, to milk the first few streams on the ground, is good. And in reply we would say decidedly yes, for not only is the number of bacteria in the fore milk much in the excess of the bacteria found in the rest of the milk, but frequently the number of species found in the fore milk is considerably larger than that in the after milk.

In reviewing the subject, there can be no doubt that the number of bacteria present in the milk as it exists before being drawn from the udder is somewhat startling, and were nothing more than an enumeration of the germs given there might be some occasion for alarm.

However, a systematic study of the germs proves that with the possible exception of rare cases, this source of bacterial life is much more beneficial than baneful to the average consumer of milk and its products.

## Elementary Medical Micro-Technique for Physicians and Others Interested in the Microscope.

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### XII.

#### BLOOD EXAMINATIONS FOR THE MALARIAL PARASITE.

Clean a finger or lobe of the ear thoroughly with soap and water, alcohol and ether. Dry and puncture with the blood lancet. Collect a small drop of blood on a clean cover and immediately mount it on a clean slip, blood side down, as a fresh preparation. The drop of blood should be rather small and mounted quickly so that a thin layer of cells may be observed. The malarial organisms if present may be seen.

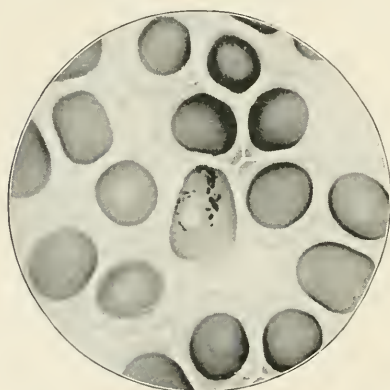


FIG. XXI.

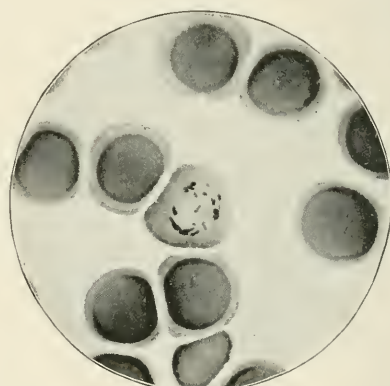


FIG. XXII.

FIGS. XXI, XXII.—*Plasmodium Malariae*. Stain special hæmatoxylin. Magnified 1800 diameters. Bausch & Lomb  $\frac{1}{8}$ -in. oil immersion objective; Bausch & Lomb compensating ocular No. 2.

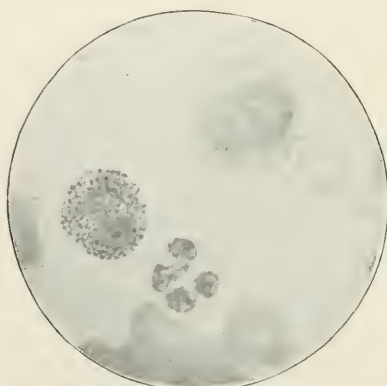


FIG. XXIII.—Blood Lymphatic Leukemia. Ehrlich's triacid stain, showing large mononuclear, polynuclear and neutrophilic corpuscles. Also red corpuscles, the relation of white corpuscles to the red being about one to eight.  $\frac{1}{2}$ -in. oil immersion objective; Zeiss projection ocular No. 4.

Jenner's stain gives good results. Apply as directed in staining blood. The malarial parasites will stain blue.

Thionin is a reliable stain for the organisms of malaria. It is used as follows: Spread a thin film of blood on a clean cover glass. Immerse it for one minute in 10 c. c. of 95 per cent. alcohol to which 1 drop of formalin is added at the time of using. Rinse the cover in water, dry between filter papers and stain for fifteen seconds in

50 per cent. alcohol saturated with thionin	-	-	20 c. c.
2 per cent. solution of carbolic acid in distilled water			100 c. c.

Wash off excess of stain. Dry between filter papers and mount in balsam as usual. Intracellular clear bodies representing an early stage of the development of the parasite are found in all forms of malarial fever. The clear space seen in many red corpuscles due to the biconcave shape of the cell should not be mistaken for the malarial organism. In tertian fever a few hours after the chill the ring appears. Pigment usually forms. The rosette and various other forms of the parasite are well stained by these methods.

The reader is referred to more complete works for further information and a description of the various forms of the parasite.

#### SIMPLE PATHOLOGICAL TECHNIQUE.

*Collecting, Fixing and Preserving the Specimen.* In ordinary cases the observer desires to make a mounted preparation of the tissue at once and in consequence he is interested only in collecting, fixing, embedding and cutting. To insure uniform and accurate results remove but a small portion of the tissue, not larger

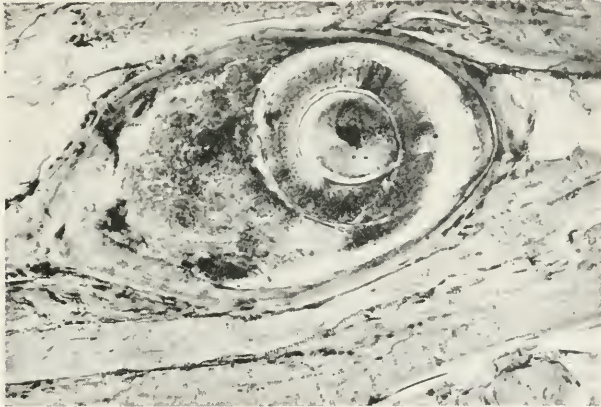


FIG. XXIV.—*Trichina* encysted in pork. Stained with hematoxylin and eosin. Magnified 150 diameters.  $\frac{2}{5}$ -in. objective; Zeiss projection ocular No. 4.

than 10 mm. square and 5 mm. thick, a much smaller piece would be better. Two or three pieces from different portions of the tissue (growth or tumor or organ) may be taken. Do not wash, but drop immediately into some good fixing solution to kill and preserve the tissue elements as in their living condition. There should be six or eight times as much fixing solution as tissue. Ordinary alcohol may be used, but Carnoy's fluid will give much better results and is more rapid in its action.

It is prepared as follows:

Absolute alcohol	-	-	-	-	60 c. c.
Chloroform	-	-	-	-	30 c. c.
Glacial acetic acid	-	-	-	-	10 c. c.

The tissue should remain in this fluid for four hours. Pour off the Carnoy's fluid and cover the specimens with absolute alcohol, which should remain half an hour. This should be poured off and fresh absolute alcohol used for a second half hour. This process washes out the Carnoy's fluid and finishes the hardening of the specimen. It is now ready to embed.

## Laboratory Outlines for the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

XXII. *Ulothrix zonata* (W. & M.) Ktz. Order, Confervales. Family, Ulothrichaceæ.

This *Ulothrix* is a small, unbranched, filamentous, green alga which usually grows in running water, attached to sticks or stones. It may be found in slow-flowing streams, in watering troughs, or in fountains. Collect the material and place it in a shallow dish in about two inches of water, and in a day or two, after the water has evaporated somewhat, large non-sexual zoöspores and sexual gametes will probably be forming. Study the fresh material and preserve some for further use.

1. Mount some of the filaments containing the basal cells (holdfasts) and study under low power. Draw.

2. Under high power draw the holdfast, the terminal cell, and two or three of the central cells; showing the wall, the chloroplast, and the nucleus. Describe these parts.

3. Non-sexual zoöspores. Examine a filament in which the cells are forming either one, two, or four zoöspores each. Observe how they escape by a lateral opening in the cell-wall. Draw and describe. These spores have four flagella and a pulsating vacuole. Draw an empty cell.

4. Sexual reproduction. Study a filament in which the cells have developed a large number (eight, sixteen, or thirty-two) small gametes of equal size (planogametes). Draw part of the filament showing some cells empty and some with gametes. The gametes have only two flagella.

5. Observe the conjugation of the gametes to form zoözygospores. Draw and describe. In order to bring out the flagella more clearly, stain with iodine solution. If the gametes do not conjugate some may round themselves off and become resting spores. This is a case of parthenogenesis.

6. Note. When the zygosporer germinates it does not develop a new filamentous plant, but gives rise to number of cells which develop as non-sexual zoöspores, and these escape and produce the filamentous plant. *Ulothrix*, therefore, along with many other thallophytes, has what is known as an alternation of generations.

XXIII. *Fucus evanescens* Ag. Class, Phæophyceæ. Order, Fucales. Family, Fucaeæ.

This brown alga is common along the Atlantic coast. It may be obtained from dealers in botanical supplies, and preserved in alcohol or other solutions. Various species of *Fucus* may be found fresh at fish stores in large cities, these plants often being used as packing. The thallus is a large, flat, dichotomously branching frond of a dark brown color, attached to various objects by means of a disc-like holdfast.



1. Place the plant in a plate of water and draw the large thallus. Describe. Note the holdfast, the flattened dichotomous frond, and the thicker central region forming a sort of midrib. Note also the swollen tips of the branches (receptacles), covered with numerous dot-like projections.

2. Find the growing points of the thallus in branches which do not have receptacles. Note the emarginate apices which have a slight groove lying in the plane of the thallus. Draw under low power. The initial cells are at the bottom of this groove. How are the branches formed?

3. Cut thin cross sections of a branch of the thallus with a razor, mount, and examine under low power. Draw. Note the outer, denser, cortical layer, and the looser, inner region, with elongated branched filaments and much mucilage.

4. Cut thin cross sections of a receptacle, mount, and examine under low power. Note the conceptacles, cavities opening by means of ostioles on the exterior. Sketch the entire section.

5. Select a favorable conceptacle and draw, showing the ostiole, the wall of the conceptacle, the sterile hairs, the large, dark-colored oogonia of oval form, and the small yellowish antheridia situated on branched hairs.

6. Under high power draw and describe a single antheridium showing cells developing into spermatozooids. About how many sperms does an antheridium produce?

7. Draw and describe an oogonium containing the eight ripe oospheres.

8. Compare the egg and sperm cells. About how much larger in volume is one than the other?

9. If fresh material can be obtained, study the spermatozooids and oospheres after their escape from the sexual organs. Take a plant with mature receptacles from sea water and expose it to the air for several hours. Mount some of the exudation, which appears at the ostioles of the conceptacles, in sea water, and examine under high power. Notice the large spherical oospheres and the small motile spermatozooids. Study the process of fertilization, and describe. Draw an oosphere surrounded by spermatozooids. The discharge of the egg from the ovary into the water is a very unusual phenomenon in the plant kingdom. Compare with *Vaucheria* and *Volvox*.

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SECTIONING STEMS AND LEAVES OF MOSSES.—Take a strip of heavy writing paper—say  $\frac{3}{4}$ -in. wide and  $1\frac{1}{2}$ -in. long; on the middle of this spread a drop of glycerine so as to cover a space about  $\frac{1}{2}$ -in. long and  $\frac{1}{4}$ -in. wide; put the part to be sectioned on this space, the end to the right; place the paper on the stage of a dissecting microscope and clamp it fast. With a pair of curved forceps in the left hand to steady the part, and with a sharp scalpel in the right, commence the cutting, watching the process through the lens. When a sufficient number of sections have been made, scrape them with the dull blade of a penknife to a dry part of the paper. If carefully done, the sections will adhere to the blade, and may be easily transferred to a slide on which a drop of water has been placed. Pick out the coarser sections, cover with a glass cover, and the remainder are ready for the compound microscope.

It is important not to have too much glycerine, and to spread it evenly; a knife-blade answers the purpose. The scalpel should have a keen edge; a knife will do if sharp enough. The parts to be sectioned should be soaked in warm water for two hours at least, and longer if possible.—*The Bryologist*.

## LABORATORY PHOTOGRAPHY.

L. B. ELLIOTT.

Devoted to Methods and Apparatus for Converting an Object into an Illustration.

### A New Projection Apparatus for Scientific Work.

Through the courtesy of the Bausch & Lomb Optical Company I have the pleasure of describing for our readers a new model of projection apparatus, designed especially for scientific work. The original model was described by me before the American Microscopical Society at Pittsburg in July last, and the apparatus has, since that time, been improved and an attachment for vertical projection added.

In the construction of this apparatus an effort has been made to secure the utmost rigidity of the optical parts, the greatest possible facility in changing their position, without changing their relation to the optical center of the apparatus, and to permit the removal and replacement of any part without disturbing any other part, at the same time always insuring perfect centering, no matter how many times removed and returned to the apparatus.

In the projection apparatus heretofore in general use, portability has seemed to be the main feature which has been kept in view, and rigidity, convenience in working and optical efficiency have been subordinated to this one demand. In the construction of the apparatus to be described no effort has been made to produce a portable apparatus in the ordinary sense of that term, although no undue bulk or weight has been added. I think everyone who has worked with the projection lantern will bear me out in the statement that the greatest amount of time is consumed and the greatest amount of trouble is caused to the operator and the lecturer through the inability to rapidly center the optical parts of the apparatus and to retain the parts in the center, once they have been adjusted. In this apparatus the fundamental principle of a fixed optical center for all parts of the apparatus has been adopted, the only adjustment required being that to bring the source of light into the optical axis, and to separate it the proper distance from the first element, namely, the rear lens of the condensing system. To this end all the optical parts and their connections are mounted upon vertical pillars attached to heavy steel blocks, which, in turn, are mounted upon a steel bar, rectangular in section, having two inclined surfaces accurately planed on its upper side, the whole contrivance resembling a fine lathe bed in rigidity and accuracy of centering. A T slot is milled in the upper portion of the rod from one end to the other, and in this a T piece attached to a vertical axis passing through the block and carrying the optical parts, is placed. The T piece may be rotated through  $90^\circ$  by means of the lever A, Fig. 1, placing its long axis parallel with the axis of the T slot, when the whole block may be lifted off from the bar, or if removed may be replaced upon the bar and held in position by releasing the lever A, which is actuated by a

spring, causing the long axis of the T piece to assume a position at right angles to the axis of the T slot. This lever, being actuated by a spring, automatically locks the block on the rod, preventing accidental overturning during adjustment. The block, with whatever optical apparatus it may carry, now rests upon the two inclined surfaces of the bar, and may be slid along its length, permitting whatever adjustment is required, and when in proper position the lever B is depressed, locking the whole rigidly upon the bar by means of a cam which draws the T piece firmly against the top of the T slot. It will thus be seen that any part of the optical equipment can be removed from the apparatus, or replaced, by releasing the T piece through the operation of the lever B, and rotating the lever A through  $90^\circ$ , and that each

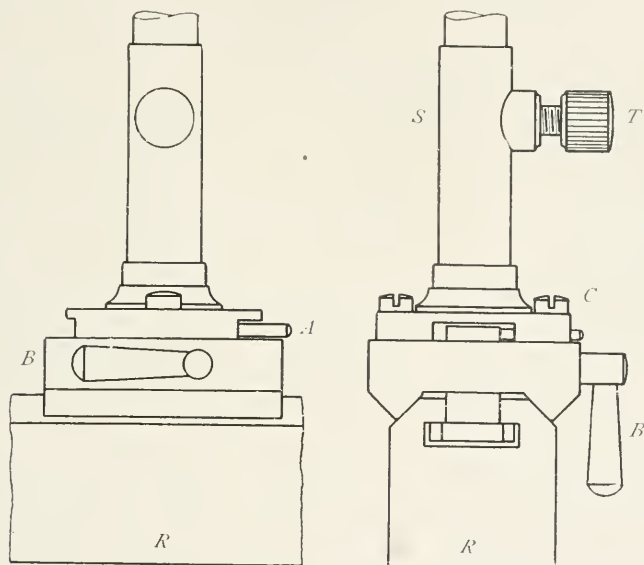


FIG. 1.—Detail of construction of base blocks for apparatus supports showing the two inclined planes on which the blocks rest. *S* piece in *T* slot which, when rotated  $90^\circ$  by the lever *A*, permits the removal of the base block from the rod; also the clamping lever *B*, which clamps the base block rigidly on the rod *R*.

element will always return exactly in the optical axis, since its support rests only on the two inclined surfaces of the rod *R*, and must in every case find the true center through the clamping action of the cam lever *B*. The rigidity of the steel bar *R* and the heavy construction of the base blocks and vertical supports of the optical part retains the alignment and centering. Adjustment is made for the original centering of the optical parts in the vertical plane by mounting them on rods which slide in the sleeve tube *S*, and when centering has been accomplished further motion is prevented by the thumb-nut *T*, which is clamped tightly, and which should, therefore, never be touched unless the part is decentered through some accident. In order to make all parts convenient for manipulation and interchangeable for different classes of work, the bellows, lens

board, slide carrier, etc., are attached to rectangular metal frames by two pins which slip into corresponding holes in the lower portion of the accessories, and by means of a spring catch with corresponding post in the upper side, thus by simple release of the knob K, Fig. 2, any of these parts can be instantly detached from their supports.

The Condenser System for collecting the light and focussing it upon the object has received special attention, and a new condenser system devised, consisting of a meniscus convex lens which is placed next to the radiant, its concave surface facing the light. By this means a greater number of rays are collected and passed forward than in the case of condensers having a plano convex lens next to the light, and a flatter field, with the least chromatic aberration, is also secured. In combination with this meniscus convex lens are two plano convex condensing lenses with their convex surfaces facing and separated by a consid-

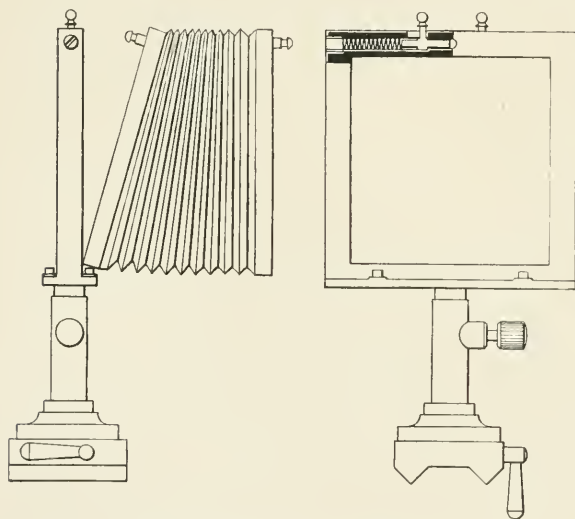


FIG. 2.—Detail of construction of supports for bellows, object, lens, board, slide carrier, etc.

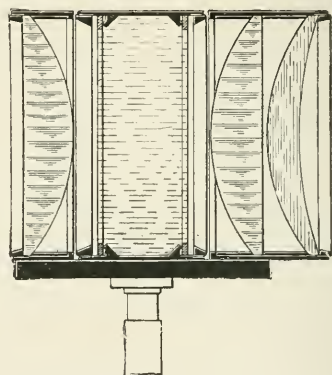


FIG. 3.—Section of condenser and water cell. The condenser is a triple system between the two anterior elements of which the water cell is placed, securing the maximum absorption of heat rays with the minimum loss of light.

erable distance. This is made possible through the use of the meniscus convex lens in the rear of the combination, and its object is to allow the placing of a water cell for absorbing heat rays between these two lenses, as, in this position, the light rays pass through nearly parallel, producing no distortion of the field and practically no loss of light through reflection from its surfaces. The water cell is composed of ground and polished glass sides mounted in a metal ring rendered water-tight by a rubber packing ring held in position by a screw ring on either side. These rings are removed when desired in order to clean the glass plates by means of a key, which renders their removal a very easy matter, thus allowing the cell to be kept always in good condition. The cell is kept in a vertical position when placed upon the table by means of two small metal feet which bear upon the table and is filled through a circular aperture at the top into which two tubes are screwed, allowing for expansion of the fluid as it becomes warm, and also permitting the attachment of two rubber tubes so that a stream



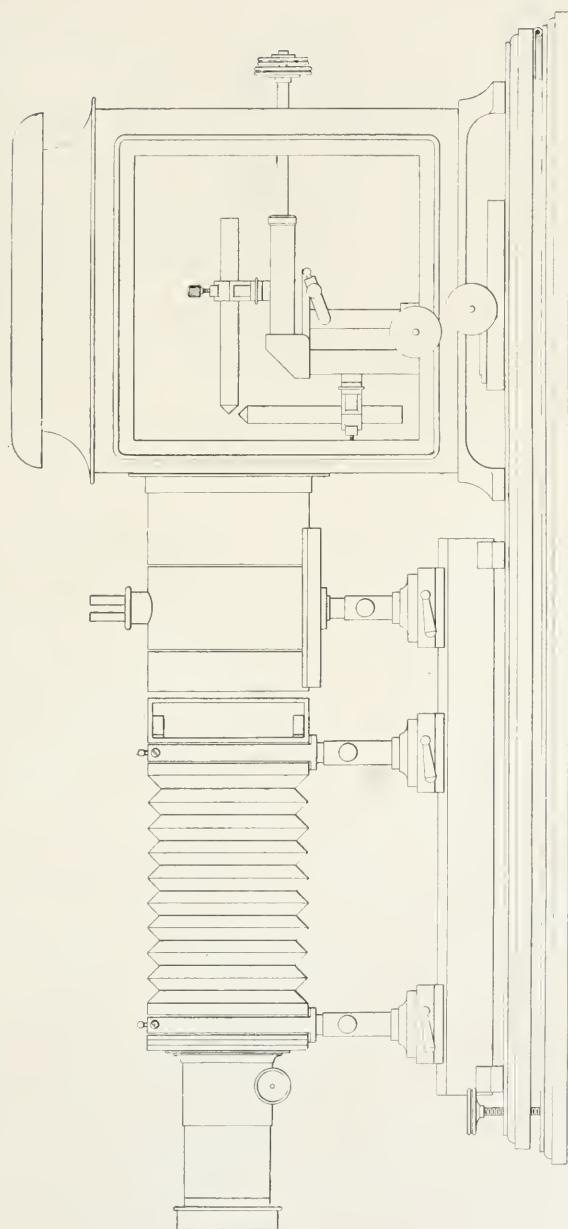


FIG. 4.—Complete Projection Apparatus with Hand Feed Lamp and Projection Lens.

of cold water can be carried through the cell when projecting living forms, if desired. In order, however, to obviate the necessity of this procedure, for ordinary work, two water cells are furnished, and when one becomes warm it is only necessary to lift it out and set another in its place. Fig. 3 shows the construction of the condenser system and water cell in detail. Fig. 4 shows the parts assembled for the projection of lantern slides. The steel bar carrying the optical parts is mounted upon a base-board which is hinged to a wooden support at the rear end, and provided with elevating screws in front, so that the circle of light may be centered upon the screen with the greatest facility. The projection lens is carried upon the first support, to the front of the iron frame of which it is attached by the catch and pins before described. The bellows connects the lens with the support carrying the object carrier to prevent the escape of light into the room, and to the rear of the object carrier is found the condenser system with water cell. This water cell is a very great advantage even in ordinary projection work, owing to its absorbing a large part of the heat, and thus preventing the cracking of valuable slides, which occurs continually unless some such precaution is used. At the rear of the condenser system we find the lamp hood, of Russia iron, with door at the side and rear, open space at the bottom and hood at the top, by which ventilation is secured to prevent heating.

The illuminant may be either acetylene, oxy-hydrogen, lime light, or electric arc. The two former lights have not yet received much consideration, but a very convenient hand feed electric arc lamp has been devised. This lamp consists of a vertical support on which two arms at right angles are carried, the vertical arm carrying the vertical carbon, and horizontal arm carrying the horizontal carbon in the optical axis. Each carbon carrier is moved by a quick acting screw, the horizontal screw being actuated by a non-conducting milled head projecting through an opening in the rear door of the lamp-hood, thus being operated from the outside when the door is closed. The vertical carbon is actuated by a similar milled head slightly smaller in size immediately back of the milled head actuating the horizontal carbon, and connecting with the vertical carbon by means of a horizontal shaft which traverses the length of the shaft operating the horizontal carbon and connecting with the vertical quick acting screw by means of a mitre gear, thus both carbons can be moved simultaneously by placing the fingers upon the two milled heads, or each carbon can be moved independently by actuating its respective milled head, at the rear, the condition of the arc being observed through a dark-glass window in the side door. Vertical motion of the whole lamp is accomplished by means of a non-conducting milled head placed beneath the vertical carbon, and easily accessible, lateral motion being effected by another milled head in close proximity to that by which the vertical motion is secured. The whole lamp is carried on a slide piece by which it can be moved backward and forward in the optical axis. A thumb-nut is provided for holding it rigidly in position when the desired focal point has been secured, thus with projection lenses of different foci, or for altering the focal point of the condensing lenses in various physical experiments, it is desirable to alter the distance of the arc from the rear condensing lens. This is very easily accomplished with-

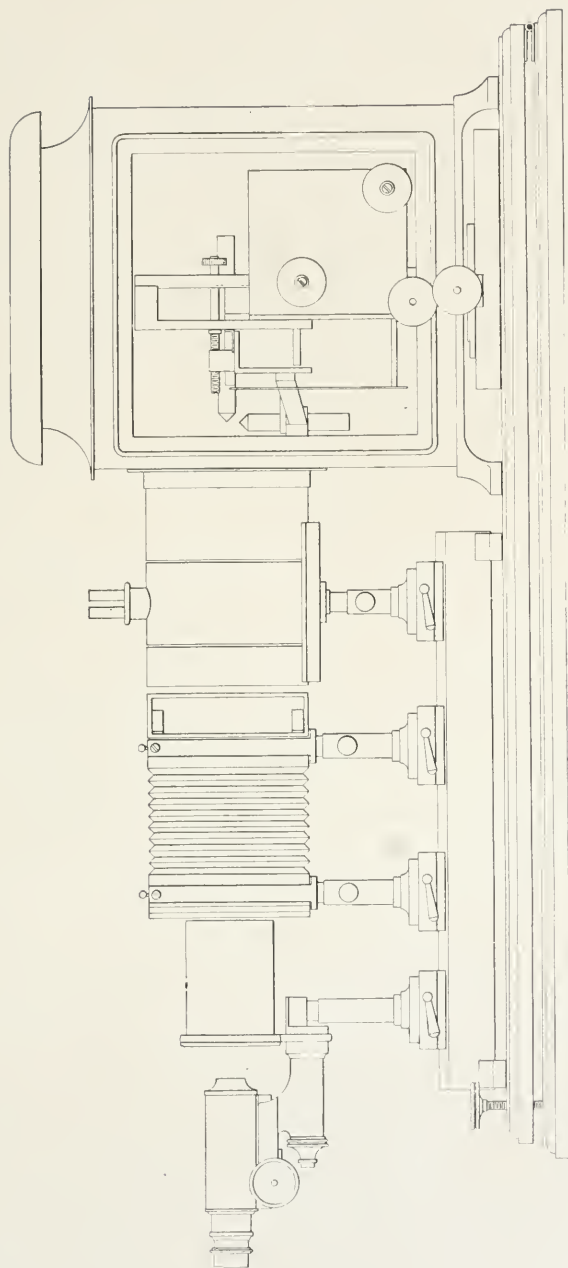


FIG. 5.—Complete Projection Apparatus with Microscope Automatic Arc Lamp.

out decentering the arc, by this means, and it is a very quick and valuable method of adjusting the illumination. The placing of the carbons at an angle of  $90^\circ$  to each other with the horizontal carbon in the optical axis, not only throws a greater volume of light from the crater of the positive carbon through the condensing lenses, but retains the glowing crater always exactly in the optical axis. No matter how irregularly the two carbons may burn, so long as the arc passes between them, the point of greatest intensity for light radiation will be in the optical axis. Any desired current can be used with a hand-feed lamp of this character, as there are no coils or magnets connected with it.

An automatic electric arc light is also in process of construction in which the carbons are placed at an angle of  $90^\circ$  to each other, the horizontal carbon being placed in the optical axis. Experimental tests with this lamp so far conducted show that it will burn without attention for at least three hours, and that, its period of regulation being very short, the variations in potential in the arc are also very slight, hence the intensity of the arc remains practically unaltered, and the flickering, hissing and other irregularities observed in lamps in which a considerable length of time elapses between the movements of the carbons seem to be almost entirely absent. This lamp is arranged to adjust vertically, laterally and horizontally, just as the hand-feed lamp does, and while it is constructed to operate on 110 volt direct current and gives good results with 10 or 12 amperes, the automatic feed may be entirely cut out and the lamp operated as a hand-feed lamp with independent movement of each carbon, on either direct or indirect current, and with any desired voltage.

The crucial test of the whole apparatus, however, lies in the substitution of the projection microscope, as in Fig. 5, for the ordinary projection lens. In this case, where microscope objectives are used, in which the front lenses are from 1 to 2 or 3 millimeters only in diameter, the whole optical apparatus must be absolutely centered and the radiant placed exactly in the optical axis in order to obtain good results. The construction proves itself accurate to such an extent that it has been possible to build upon one of the supporting bases a double projection apparatus, consisting of two arms at right angles attached to a common center, one bearing the ordinary projection lens for projecting lantern slides, and the other a projection microscope, the construction being shown in Fig. 6. It will thus be seen that either the projection microscope or projection lens can be swung into the optical axis and the accuracy of the centering and stability of the whole is such that when the projection lens is focussed upon an object in the slide carrier, and the projection microscope is focussed upon an object on the microscope stage, the light being suitably arranged for either, the other may be swung into position, and its image instantly projected upon the screen; thus, if the demonstrator is desirous of showing, for example, an illustration of hydra, representing it in its living form, with its surroundings, he can do so by means of a lantern slide and instantly substitute upon the screen for this picture the image of a cross section of the hydra itself by means of the projection microscope. Any part of a lecture can be illustrated with lantern slides and microscopic sections can be interspersed between them. The value of this attachment permitting the use of the projection microscope or the ordinary projection lens at will, will



be more appreciated when one has an opportunity to run through a long series of papers on different subjects, such as are found on the programs of society meetings or in the lecture room in a large educational institution during a day's work. The projection microscope used with this apparatus is of very simple construction, being simply a microscope fine adjustment and coarse adjustment with body tube and stage, as in an ordinary microscope, but instead of the pillar and base a special arm which adapts it to one of the supporting blocks identical with those which carry the other optical parts. The stage is provided with a sub-stage actuated by quick acting screw, in which condensing lenses of various foci can be used for different power objectives. The microscope lenses employed for projection work with this apparatus consist of microscope objectives having a somewhat less numerical aperture than those usually employed for visual pur-

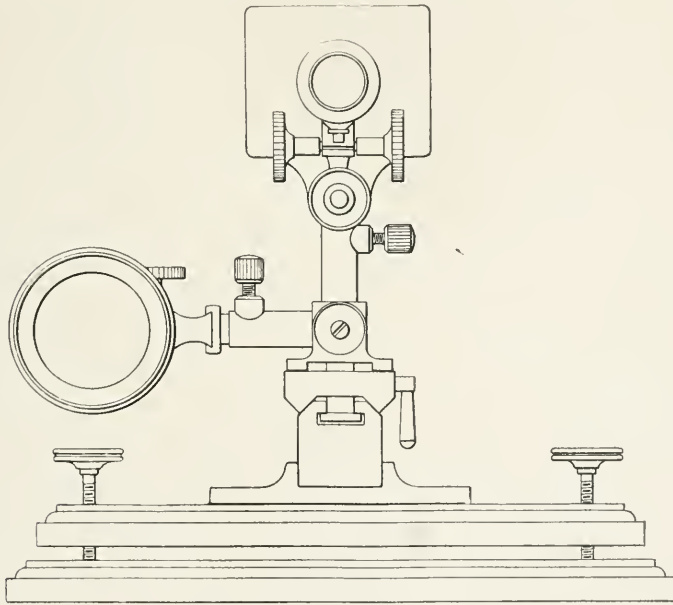


FIG. 6.—End view of combined slide and microscope object projector.

poses, thus securing greater depth of focus or penetration, increasing their value for thick objects, and especially corrected to eliminate spherical aberration and color, without special regard to chemical focus, as this is of no consequence in a projection lens. The mounts are perfectly straight and provided with a sliding hood carrying a diaphragm. The size of the aperture in this diaphragm is regulated to cut off the edge of the field, so that a perfectly sharp and well defined circle appears on the screen, as (without this diaphragm) the image would gradually fade off, giving an unpleasant effect. When, however, it is desired to increase the size of field for regional demonstrations, etc., it is only necessary to slip the hood off from the objective and replace it again when needed. Powers of from three inches down to one-half inch are used with great facility on ordinary screens, at the ordinary distance at which ordinary projection lenses are used, giving ample illumination for observation, even in the rear

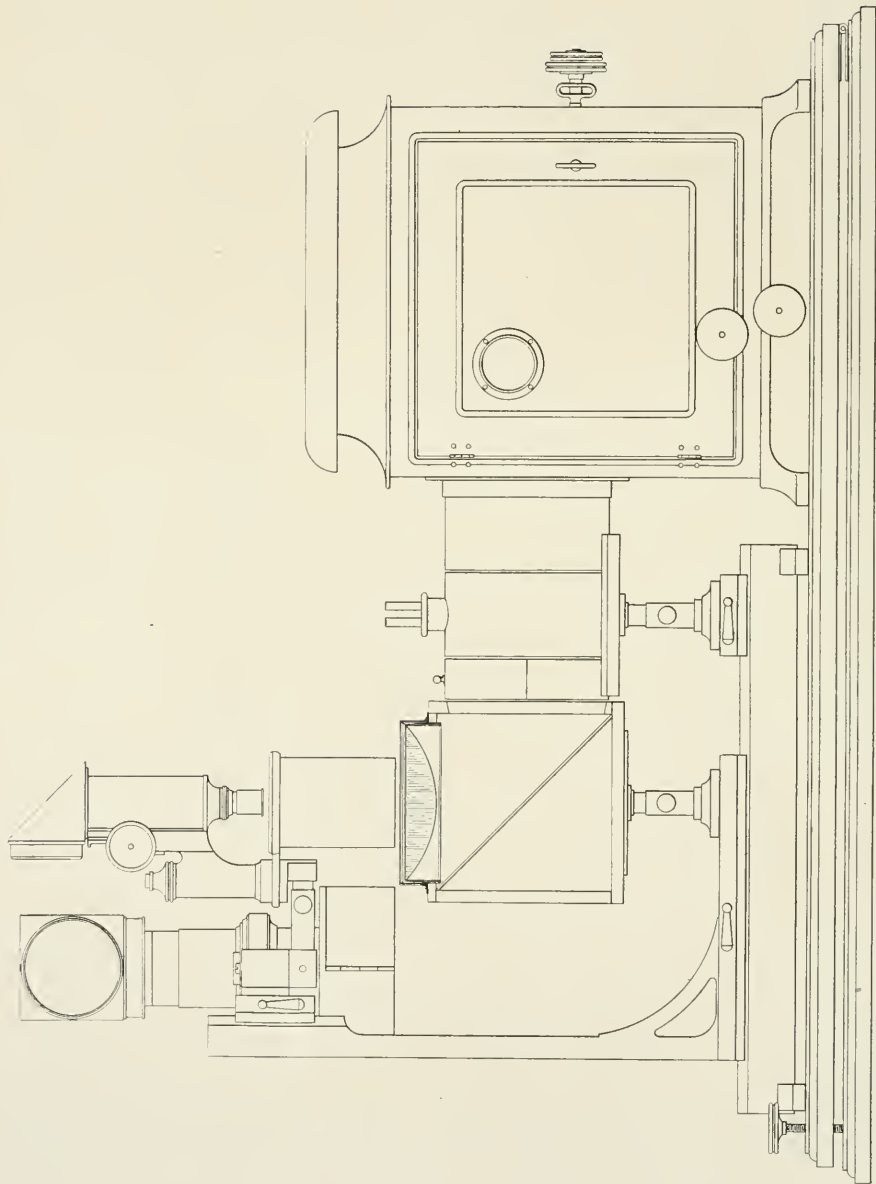


FIG. 7.—Vertical Attachment for Complete Projection Apparatus.

of a good sized room, and with circles up to eight or nine feet in diameter. When, however, it is desired to use higher powers, the best results are obtained by using smaller circles, perfectly opaque and perfectly flat, white screens, or better still, semi-transparent screens for the very highest powers, and placing the lantern facing the audience, projecting the image so that it will be viewed through the screen. In this way it has been possible to show bacteria with a  $\frac{1}{12}$ -inch oil immersion lens to audiences of considerable size.

The apparatus, as thus far described, is intended for projection of such objects as can be stood upon edge and projected in the ordinary manner, preferably permanent microscopical preparations and ordinary lantern slides. Where, however, it is desired to show living forms under the microscope, or under the ordinary projection lens in fluid, etc., an apparatus for diverting the beam from the condensing lenses to a vertical path, and then bringing it again to the horizontal axis, is required. For this purpose a horizontal attachment to the projection apparatus has been devised, a diagram of which is shown in Fig. 7. This attachment is intended for use with either the projection lens or projection microscope. It consists of a large plane mirror with a very perfect surface set at an angle of  $45^\circ$ , mounted in a light-tight box and carried on a rectangular arm. This is attached to the base bar of the projection apparatus by a T piece, as the other attachments are. The vertical arm for this attachment is arranged to hold the microscope and projection lens used for regular projection, and, when placed upon the arm, the microscope and projection lens come into the optical axis of the beam of light projected upward from the mirror. For use with the projection lens, the front plano convex condensing lens is lifted out of the condenser system, it being mounted in a ring with knob for this purpose, and placed with its plane surface uppermost in the cell above the inclined mirror. A plane plate of glass mounted in a suitable ring is then placed over the condensing lens to protect it, and the objects to be projected are placed upon this glass plate. The projection lens is focussed upon it, and upon the hood of the projection lens a  $45^\circ$  prism is placed which diverts the vertical beam to a horizontal path, throwing the magnified image upon the screen. A small percentage of light is lost in these reflections, but where the apparatus is perfectly centered, and the prism and mirror surfaces are very accurately made, the percentage is so small that with the arc light no difficulty is experienced in projecting lantern slides and all ordinary translucent objects at ordinary distances, and securing circles of the same size as with the ordinary projection lens. At about twenty feet distance, with the one-fourth projection lens, magnifications of about 30 diameters are had, and an object over four inches in diameter can be shown, so that one can take a four-inch Petri dish for example, filled with water, and place in it a series of frog larvæ from the egg up to forms with legs and demonstrate the whole series at one operation. The formation of crystals, the observation of dissections and hundreds of other uses will suggest themselves for an apparatus of this kind. When the projection microscope is swung into position, the plano convex condensing lens is returned to the front of the condenser system, and its protecting plate is replaced by a

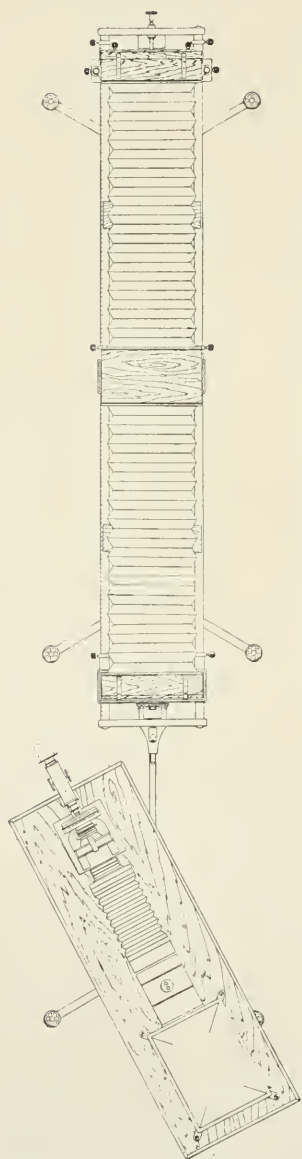


FIG. 9.—Top view of Projection Apparatus and Photo-micrographic Camera showing Projection Apparatus swung out of the optical axis for focusing and arranging object in the field.

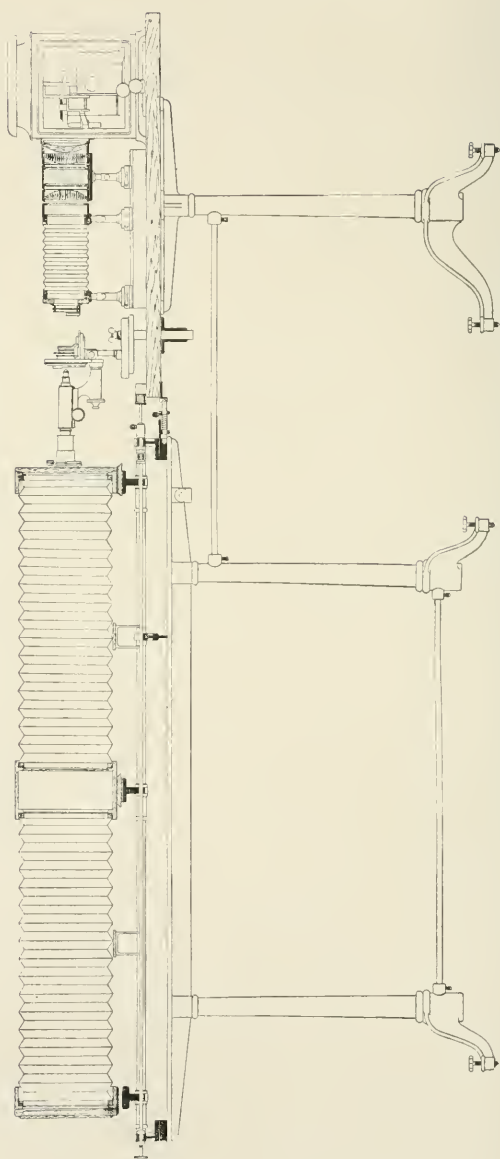


FIG. 8.—Complete Photo-micrographic Camera and Projection Apparatus.



metal diaphragm having a circular opening of the proper size to admit the beam of light from the mirror into the condensing system of the microscope. A  $45^\circ$  prism placed upon the top of the microscope tube serves the purpose of reflecting the vertical beam to a horizontal path. With the projection microscope the illumination is sufficient for use with powers up to and including one-half inch with ordinary screens at the same distance that the projection lens is ordinarily used, and with undiminished circles of light.

The great advantage of the horizontal position for preparations of living forms such as *Paramœcium*, *Gammarus*, *Entomostraca*, *Amœba*, *Vorticella*, and other infusorial forms, as well as any other microscopical thing in fluid which it is desired to show, as, for example, urinary deposits, crystals, etc., is apparent.

The optical bench, as above described, is mounted upon a three-legged iron support so that it may be rotated completely around, and this mounting can be used for photomicrography as well as for projection work, being in this case attached to the complete photomicrographic camera as shown in Fig. 8., the microscope being swung out of the optical axis when it is desired to arrange the object in the field and for preliminary focussing, as shown in Fig. 9. This construction can be taken advantage of in the lecture room by having the photographic dark-room situated at the rear of the lecture room provided with a window opening onto the lecture room. The camera and optical bench can be lined up at the side of the dark-room next to the lecture room and the whole apparatus used for photomicrography when desired. When the optical bench only is desired for projection, the window can be opened, the optical bench turned through  $90^\circ$  so as to project its image upon the screen, and both vertical and horizontal attachments can be used without difficulty in this manner. This projection apparatus can also be used to great advantage for making bromide enlargements, both from lantern slides and from microscopic objects to be used as charts. Any points to be emphasized can be worked up on such enlargements with crayon or pencil after the enlargements have been made, and any portions can be colored with analin or water colors. This is a field of work which such a projection apparatus will greatly facilitate, and one which has as yet been very little developed. It is believed that this construction will lend itself particularly well to physical and chemical demonstration work, and the special accessories for these uses which are now in process of development will form the subject of another paper.

L. B. ELLIOTT.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

**Strasburger, Edward.** Ein Beitrag zur Kenntniss von *Ceratophyllum submersum* und phylogenetische Erörterungen. Jahrb. f. wiss. Bot. **37**: 477-528, pls. 9-11, 1902.

This important paper gives a full account of the morphology and biology of *Ceratophyllum* and also presents a discussion of the factors concerned in

constructing a natural system of plants.

*Ceratophyllum* is pollinated under water. An enormous quantity of pollen is produced, of slightly higher specific gravity than water. The stigma has a peculiar shape well adapted to catching the pollen, so that while the loss is great, few ovules fail to produce seeds. Double fertilization was observed and the number of chromosomes—twelve in the gametophyte and twenty-four in the sporophyte—was counted. At the first division of the endosperm nucleus a wall is formed dividing the embryo-sac into two chambers, of which the one nearest the chalaza does not divide again. The other divides transversely, and here also only the one next the micropyle divides again. After a few such divisions, the cell remaining next the micropyle gives rise to a small celled tissue. The embryo has no suspensor and no primary root. The embryo so closely resembles that of *Nelumbo* as described by Lyon, that Strasburger examined *Nelumbo* and decided that here also there are two cotyledons and not one, as Lyon claims.

The philosophical portion of the paper contains a discussion of the criteria of homologies, the role of mutation, external influences, natural selection and recapitulation as they are applied in building up a phylogeny. C. J. C.

**Land, W. J. G.** A Morphological Study of *Thuja*. Botanical Gazette, **36**: 249-259, pls. 6-8, 1902.

Ovules up to the stage of formation of archegonium initials were fixed in the usual one per cent. chromo-acetic acid

solution, but for later stages this reagent penetrates too slowly. Carnoy's formula was better for fertilization and succeeding stages. Although many fixing agents were tried, none proved entirely satisfactory. Haidenhain's iron alum-hæmatoxylin is recommended for staining, except in case of embryos, where Delafield's hæmatoxylin followed by orange G gave better results.

The pollen tube structures and the development of archegonia are described in detail. A definite ventral canal cell is not formed although the nuclear division takes place. The writer believes that the ventral canal cell is represented, at least by such a nucleus, in all the Coniferales. This nucleus often divides so that the upper end of the archegonium sometimes looks like a young proembryo. The fertilized egg gives rise to eight free nuclei before cell walls begin to be formed. C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

**Hesse, Fried.** Zur Kenntniss der Granula der Zellen des Knochenmarkes, bez. der Leucocyten. *Anat. Anz.* **18**: 452-461, 1902.

This work gave the author the chance of testing Ehrlich's theory of granular leucocytes. The theory in brief is,

that granular leucocytes form an important group of white blood cells. These granular cells are divided according to the granules found in the protoplasmic body. The kind of granule is determined by the use of a definite fixation (heat) and staining process (triple stain). It is axiomatic that the staining process with dry preparations is due to a chemical change. Different granules stain differently owing to their chemical natures and are recognized as basophile, acidophile, neutrophile, amphophile, forming definite series without transition forms. More than this, certain forms of granules are found to be characteristic of certain animals and in others another kind is their equivalent. Such are the pseudo eosinophile granules in the rabbit with the corresponding neutrophile form in man. The author used for his studies dry preparations of bone marrow from the rabbit, which was treated exactly according to Ehrlich's process. The dry preparations were fixed either in ether-alcohol or alcohol at 100°, 120°, 140° C., treated with glycerine and aqueous staining solutions of eosin, indulin, aurantia, orange G., with solutions of eosin-indulin-glycerine, Erlich's triple glycerine mixture, methylen-blue or dahlia, or else after first staining in eosin or aurantia counterstaining with methylen-blue or with triacid. Lastly, Laurent's eosin-methylen-blue with anilin oil, xylol differentiation. The following results were obtained from rabbit bone marrow: (1) Both in similar and different cells and within a single cell there are wide variations in eosinophile and pseudo-eosinophile granules as to size, form, refraction, and number. (2) There is a difference in intensity of staining of the granules within a single cell on the use of a single stain as well as one produced by the use of a mixed stain. (3) In equivalent cells there is frequently a difference of stain of the intergranular protoplasm. (4) Temperature causes reagents to act differently on granules of different cells and those in a single cell.

The author's conclusions are: (1) That Ehrlich's classification of leucocyte granules dependent on color-analysis is undemonstrable. (2) There are not only differences of granule stains according to the reagents and processes used and different kinds of granules within a single cell, but transition forms can be found. (3) The dependence of micro-chemical reaction of many granules upon the definite experimental or pathological processes to which leucocytes are subject, and upon the changes due to karykinesis account for some of the differences. (4) Since, on account of transition forms, it is impossible to make a classification of leucocytes according to differential staining, the conclusion is necessitated that the granular leucocytes are all of one nature, but possess an extraordinary power of repair and adaptability, especially in regard to the granules contained within the cells. (5) Evidence is strongly in favor of the conclusion that these granules are essential structural parts of the cells and have a far-reaching influence on resorption, assimilation and secretion.

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

Van Wijhe, J. W. Eene nieuwe Methode ter Demonstratie van Kraakbeenige Mikroskeletten. Versl. d. Wis. en Natuurk. Afd., 834-837, 1902.

The author has made successful preparations of the cartilaginous skeleton of *Amphioxus*, of the embryos of the shark and ray, of the salmon and roach, of the frog, lizard, bird, mouse, rabbit, and man. Most basic anilins have an affinity for cartilage and methylen blue was selected after experiment as the most satisfactory for use in this method. The embryos were usually fixed in 5 per cent. sublimate to which  $\frac{1}{10}$  vol. of formol is added before using. Other agents such as 10 per cent. formol, Zenker's fluid, or simple alcohol gave good results. Even partially disassociated embryos were utilized. When iodine-alcohol is used after sublimate it is advisable to get rid of the iodine, which otherwise forms an insoluble precipitate with the methylen blue. It may be removed by alcohol containing  $\frac{1}{4}$  per cent. HCl renewed each day until no trace of yellow tint forms in the alcohol. The embryos are stained in alcoholic methylen blue prepared as follows: alcohol 100 parts, HCl 1 part, methylen blue  $\frac{1}{4}$  part. The acid causes the formation of needle-like crystals and should therefore be added some time before using the stain. The stain is allowed to act for 1 to 7 days and is then washed out in the above mentioned acid alcohol until stain is no longer extracted by the decolorizer. There is no danger of over-extraction. The cartilage in embryos left in acid alcohol for over a year remained a bright blue. A week suffices for most embryos, and, at the close of the process, all tissues but cartilage should be colorless. The process may be hastened by alternating 70 per cent. and a stronger alcohol. Dehydration is followed by a gradual transfer from absolute alcohol to xylol, by thin and then thick balsam in xylol and by inclusion in balsam which becomes liquid at 60°, this last step being completed in a thermostat. Permanent mounts are made in this medium in built up glass cells. The author uses only the solid neutral Canada balsam of Grüber to avoid the turpentine found in commercial solutions. Preparations in balsam for two years have shown no tendency to fade. Macroscopic museum preparations of shark embryos 20 cm. long, preserved in xylol were exceedingly beautiful at first, but later the non-cartilaginous tissues lost their transparency and became opalescent.

C. A. K.

Prenant, A. Notes cytologiques: VI. Formations particulières dans le tissu conjonctif interstitiel du muscle vésical du Brochet. VII. Contribution à l'étude de la ciliation de la partie adhérente du Myxidium lieberkühni. Arch. d' Anat. Micr. 5: 191-213, pl. ix, 1902.

Many standard fixing agents were used, but the best results were obtained after Perenyi's fluid and the formol-picric mixture of Bouin. A very satisfactory triple stain was obtained by following Heidenhain's iron hæmatoxylin with methyleosin or with erythrosin. The surplus stain is washed from the slide with water and the sections are treated with Lichtgrün in strong aqueous solution. Nuclear chromatin and other structures retaining the hæmatoxylin lake retain their deep black color, the protoplasm of the smooth muscle fibers has a rosy tint, while all intermuscular connective tissue is green. The author commends it highly as a general differential staining method.

C. A. K.



## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory,  
University of Michigan, Ann Arbor, Mich.

**Lohmann, A.** Untersuchungen über die Verwerthbarkeit eines Delphininpräparates an Stelle des Curare in der Muskelphysiologischen Technik. Arch. f. d. ges. Physiol. 92: 473-478, 1902.

The author suggests as a useful substitute for curare in work on muscle-physiology an easily soluble preparation of *delphinin*, made by G. Heyl in Darm-

stadt. The curare preparations on the market are rather uncertain in their action, and pure curarin is too expensive for general use, so that a reliable inexpensive substitute for curare is much to be desired. As the result of a series of comparative test experiments Lohmann thinks such a substitute is afforded in delphinin. One gram of delphinin to 1000 gr. of frog produces complete paralysis as a rule. The poison is injected subcutaneously in 4 to 8 per cent. solution. In about one minute the frog becomes sufficiently paralyzed so that it will remain quiet in any position, although direct stimulation of a nerve will still cause muscular spasms. Experiments showed that the delphinin had no effect on the form of the contractions, nor was the magnitude of the contractions of the poisoned muscle less than in the case of the normal muscle. Detailed descriptions of the action of the drug are given in the paper. The preparation of delphinin recommended may be obtained from E. Merck, Darmstadt.

R. P.

**Forsell, G.** Ueber die Bewegungen in Handgelenk des Menschen. Skand. Arch. f. Physiol. 12: 168-258. Taf. v-viii, 1901.

An extensive, detailed study by means of Röntgen rays of the movements of the bones of the wrist. Full directions

for the use of this method for studying the movements of joints are given.

R. P.

**Kuliabko, A.** Studien über die Wiederbelebung des Herzens. Arch. f. d. ges. Physiol. 90: 461-471, Taf. III, 1902.

Neue Versuche über die Wiederbelebung des Herzens. Wiederbelebung des menschlichen Herzens. Centralbl. f. Physiol. 16: 330-331, 1902.

By means of an artificial circulation of Locke's solution, warmed to body temperature, through the isolated heart, Kuliabko has been able to obtain extraordinary results in causing a renewal of activity in the mammalian heart

after its apparent death, and at considerable intervals of time after the death of the animal. As an example of his results an experiment described in the earlier of the two papers may be mentioned. The heart of a rabbit was removed and an artificial circulation of Locke's solution at a temperature of 40°C. was kept up through it for about an hour. The heart beat regularly and strongly during this time. At the end of the hour the current of fluid was stopped and the heart with the attached cannula was placed in an ice-chest where the temperature was practically 0°C. In five minutes all pulsation had ceased. After the heart had remained on the ice for 18 hours it was removed and again connected with the circulation apparatus. In less than a half minute after the circulation

began, strong, rhythmical contractions in the region of the openings of the venæ cavæ appeared. This pulsation soon spread till it included all the heart except the left ventricle, which in this particular experiment did not regain its activity. A number of experiments similar to this are described in detail, and an excellent review of the literature of the subject is given.

In the second of the papers the author reports a continuation of these experiments. He has been able to bring about, by the same method as that used in the earlier experiments, a re-appearance of pulsations in rabbit and bird hearts after periods of total inactivity of as long as three, four and even five days. These results were obtained not only with hearts removed from freshly killed, healthy animals, but also with hearts from animals which had died natural deaths (from disease or otherwise). This led to a series of experiments with hearts from human bodies. The heart of a boy who had died of *pneumonia duplex* was removed from the body about twenty hours after death, taken to the laboratory and flushed through the aorta with warm, oxygenated Locke's solution. In about twenty minutes parts of the heart began to pulsate and finally the movement extended over the whole organ, and the whole heart continued to beat regularly for an hour. The same results were obtained in numerous experiments with the human heart, not reported in detail in this preliminary communication. The author points out the evident practical importance of these results in cases of stopping of the heart beat and apparent death.

R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt, Harvard University Medical School, Boston, Mass.

**Moak.** On the Occurrence of Carcinoma and Tuberculosis in the Same Organ or Tissue. The J. of Med. Research, 8: 128-147, 1902.

Moak presents five cases in which carcinoma and tuberculosis were found associated in the same organ

or tissue. He found carcinoma might be the primary process and the tuberculosis secondary. In one case he observed quiescent tuberculosis of a gland which was secondarily invaded by metastatic carcinoma. In another both diseases seemed to be transmitted in the same way as metastases, and at the same time. He concludes, then, that either may appear first or both may be noted at the same time. The difference in the form of combination depends partly on the anatomical peculiarities and partly on the susceptibility of the tissue to one or the other of the diseases. In considering the supposed antagonism, he states that the following points should be borne in mind: 1. Cases of active tuberculosis occur, for the most part, at a time of life before cancer becomes prevalent. 2. If latent or healed tuberculosis be taken into account it is evident that the two diseases must be very frequently associated in the same individual. 3. The organs most frequently affected by tuberculosis are not the organs most frequently attacked by carcinoma. He is of the opinion that no real antagonism exists.

W. R. S.

**Flexner.** On Thrombi Composed of Agglutinated Red Blood Corpuscles. Preliminary Communication. Univ. of Penna. Med. Bull. 16: 324-326, 1902.

The phenomena of agglutination has been considerably studied of late and much that is new has been added to our knowledge of it. For example, Flexner and Noguchi have proved that the agglutinating principle for blood corpuscles in snake venoms is distinct from the dissolving substance. This has been observed in ordinary hemagglutination as seen in normal sera. It has also been shown that agglutination and lysis of bacteria are produced by two different groups of substances. Within the last twelve months it has been found that the products of some bacterial cultures are hemolytic, and the temperature seems to play an important part in this phenomena, for it is usually absent when the freezing point is approached. Agglutination, however, is not prevented by such temperatures.

The occurrence of thrombi, composed of agglutinated red blood corpuscles, does not seem to have been previously noted. Flexner's attention to this phenomena in bacterial disease came from his study of a case of typhoid fever which presented such a thrombus in the dilated veins of the intestinal mucosa. The mass composing the thrombus was made up of globules of different sizes showing different degrees of refraction and varying staining properties. On careful study it was found to be composed of altered red blood corpuscles. Such a thrombus was searched for in twenty additional cases of typhoid fever, but no such convincing instance as the above was found. In two other affections the thrombi were seen. Flexner is inclined to the view that the hyaline glomerular thrombi consist of red blood corpuscles. So in thrombi in other than bacterial diseases, evidence was obtained that some are similarly constituted. A case of eclampsia is cited in which they were found in the liver. He suggests that some thrombi described by other writers may be of this nature. He was able to produce non-bacterial agglutinative thrombi in animals by the injection of ricin, ether and alien globulicidal blood sera.

In his conclusions he states that these thrombi are not uncommon in infectious diseases in man and animals, that they may present, when old or when the agglutination is compact, the appearances to which the name of hyaline thrombi has been applied; that the so-called "fibrin ferment thrombi" are probably nothing else than these thrombi.

W. R. P.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn, Wesleyan University, Middletown, Conn.

**Hiss.** New and Simple Media for the Differentiation of the Colonies of Typhoid, Colon and Allied Bacilli. Jour. Med. Research, 8: 148, 1902.

Hiss has shown that it is possible by a very simple modification of ordinary culture media to differentiate the colonies of the typhoid bacillus and the colon bacillus upon ordinary plates. The media which he uses for this purpose are several in number. All of them, however, contain agar, and they are quite

similar to the ordinary nutrient media. For example, the one upon which the largest amount of work has been done, is made up of agar, 15 grams; gelatine, 15 grams; Liebig's extract, 5 grams; dextrose, 10 grams; water, 1000 c. c. This material, as will be seen, differs from the ordinary nutrient agar in hardly any point except that the *peptone is omitted*. In the other media which he describes the peptone is in a similar manner always omitted. Using these media for cultivating typhoid and colon bacilli Hiss finds the two species very readily differentiated. The typhoid bacillus produces a colony considerably smaller than the colon, and it also develops, after proper growth, an abundance of irregular filaments radiating from a central colony, whereas the colon bacillus produces a colony with a uniform outline. This filamentous condition is a very ready means of differentiation of the two types of bacteria.

H. W. C.

**Weil.** Zur Schnell diagnose der Typhusbacillen. Hyg. Rund. p. 485, 1901.

The author devises a new method for rapid diagnosis of typhoid fever as follows: A medium is prepared containing 600 grams of grated potatoes which are allowed to stand 12 hours at 15°C. This material was then pressed through cloth, and to 300 grams of it are added 200 grams of bouillon with a slightly alkaline reaction, and 3.75 grams of agar are added to the mixture. The medium is filtered and sterilized, and upon it typhoid colonies become a silvery grey and finely filamentous, at a temperature of 37°, while the coli colonies are larger, round or oval and do not show the filaments. By the use of these media the author has been able to isolate the typhoid bacilli from water, feces, etc.

H. W. C.

**Courmont, Jules.** Concerning the Presence of the Bacillus of Eberth in the Blood of Typhoid Patients and its Bearing upon the Prognosis of Typhoid Fever. Journal of Physiology and General Pathology, Jan. 1902. Bulletins and Memoirs of the Medical Society of the Hospitals of Paris, Jan. 2, 1902.

After an historical glance at the question, the author gives a few details upon its technique and upon observations made in the course of numerous investigations. We give a résumé of the conclusions deduced from this work.

The typhoid bacillus exists in a constant manner in the blood of patients who are suffering from typhoid fever in its ordinary, severe, or fatal forms; the author has no knowledge in regard to the presence in those cases where the attack is either mild or averted. The microbe appears in the blood at an early period, before the fifth day, and remains until the end of the third week. In prolonged cases, or where a relapse occurs, the bacillus of Eberth may remain still longer in the blood, or may disappear. The bacilli obtained from the blood are those of Eberth, with all their specific characteristics; they are, however, only feebly agglutinative; but this feeble power may be developed in subsequent cultures. The presence of the typhoid bacillus in the blood has no bearing upon its power of agglutination. The serum-reaction may be greatly retarded if the bacillus has been found in the blood some time beforehand. Consequently there is a very valuable method of making a preliminary diagnosis of typhoid fever when the serum diagnosis is negative. This method consists in inoculating 300 to 600 c. c. of ordinary bouillon with 2 to 4 c. c. of blood immediately after it has been taken, and propagating it in the incubator at 37°. In this way a pure culture of typhoid fever may almost always be obtained. On the contrary, the introduction of a few drops of blood into a small quantity of bouillon gives, in nearly all cases, negative results.

A. GIRAULD.

Tr. by Eleanor Larrabee Lattimore.



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Journal of

# Applied Microscopy and Laboratory Methods

Edited by L. B. ELLIOTT.

**SEPARATES.**

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WITH this number we begin our sixth year of publication, and we are pleased to be able to use for the coming year a much finer quality of paper than heretofore, and we shall also publish more pages in Volume VI than in any previous one. The increased scope of the JOURNAL enables us to publish this year material covering practically every phase of laboratory work of interest to the worker in the biological sciences. One of the most rapidly developing branches of laboratory work is that employing photographic processes, and we shall make a special effort to place before the readers of the JOURNAL during this year a wide variety of information on this subject. The series of papers by Prof. Gratacap of the American Museum of Natural History, New York, will be continued, and we are arranging for a series of collateral articles taking up special branches of museum work by men who have made a specialty of these branches. We also expect to have more in the nature of field methods and the accessory methods leading up to microscopical work.

The support of all interested in the development in America of a laboratory journal on broad lines is solicited during the coming year. The JOURNAL is not a money making venture, and all proceeds received from subscriptions and advertisements are turned into its improvement.

THE fifth annual meeting of the American Association for the Advancement of Science was held in Washington, D. C., December 29th to January 3d. A more ideal city for holding such a meeting could scarcely be imagined, and the attendance proved that the selection was a wise one, the total enrollment being 989, second only to that of the Boston meeting in 1880, when twelve more members were enrolled. In addition to this number there were also 363 members of affiliated societies who registered at the secretary's office, making the total registration of scientific men in attendance at the meetings 1352. Making a conservative estimate of those who did not enroll, there must have been at least 1500 scientific men attending the meetings at Washington during Convocation week. The increase in membership of the Association and the increase in the interest in the programs, and the character of the men who are directing the affairs of the Association since Dr. Howard became permanent secretary is very gratifying indeed. The plan formulated last year by the American Association for the concentration of all the societies of the country into one body had its first test at this meeting, and while there were occasional conflicts between several of the organizations and sections of the American Association covering the same field, the ultimate arrangements have, in nearly every instance, been satisfactory and have proven that consolidation of all societies is practical and, we think, without doubt, highly beneficial. The next meeting of the Association will be held in St. Louis during Convocation week, 1903-4. Philadelphia is recommended as the place for the meeting in 1904-5.

The officers for the ensuing year are as follows:

*President*—Carroll D. Wright, Washington.

*Vice Presidents*—Section A, Mathematics and Astronomy, O. H. Tittmann, Washington; B, Physics, E. H. Hall, Harvard University; C, Chemistry, W. D. Bancroft, Cornell University; D, Mechanical Science and Engineering, C. M. Woodward, Washington University; E, Geology and Geography, I. C. Russell,

University of Michigan; F, Zoölogy, E. L. Mark, Harvard University; G, Botany, T. H. Macbride, University of Iowa; H, Anthropology, M. H. Saville, American Museum of Natural History; I, Social and Economic Science, S. E. Baldwin, New Haven; K, Physiology and Experimental Medicine, H. P. Bowditch, Harvard University.

*Permanent Secretary*—L. O. Howard, Cosmos Club, Washington.

*General Secretary*—Chas. W. Stiles.

*Secretary of the Council*—Chas. S. Howe, Case School.

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*Treasurer*—R. S. Woodward, Columbia University, New York.

The American Association for the Advancement of Science deserves the support of everyone interested in any way in scientific work, and we should be pleased to furnish application blanks and information regarding the Society to any of our readers who desire to become members.

A business meeting of the American Microscopical Society was held on January 2nd, at the main building of the Columbian University, Washington, D. C., at which the President, Prof. A. E. Birge, University of Wisconsin, presided, the attendance being unusually large, especially as this was only a business session, no papers being presented, the secretary having previously informed the members that abundant material had been received for the 1903 Proceedings.

The principal item of business was a discussion as to whether a summer meeting of the Society should be held or not. After thorough discussion of the subject, it was decided to hold a summer meeting next summer, place and time to be decided by the executive committee. It was suggested in this connection that these summer meetings should be working meetings, and should be held at some place where plenty of material of interest can be had, such, for example, as one of the Seaside Biological laboratories, at which collections could be made during the days of the meetings, and the material distributed among the members to be worked up and described in the Proceedings; the Mammoth Cave of Kentucky, where a survey of the blind fauna of the cave could be made and form the subject of a special report, and many other suitable localities were proposed, and the sense of the meeting was that a plan of this kind would be productive of the greatest amount of good for the summer meeting. This would not interfere in any way with holding a winter meeting at which a program of papers could be arranged if desirable. This question was left open for future consideration. Another question of considerable importance to the future of the Society was also brought forward, and referred to a special committee to be appointed by the President, namely, the adoption of a plan by which an arrangement with some regular publication devoted to microscopy could be made, so that each member of the Society could receive a copy of this publication regularly, and that the Society could publish therein its announcements to members and any other information which might be of general interest to them. It was suggested that the interest of the members in the Society would be greatly increased if there was some such medium of communication whereby various occurrences in connection with the Society's work could be brought to the knowledge of the members oftener than once a year. At the close of the business session the Society adjourned to the chemical lecture room and witnessed a demonstration of the new Bausch & Lomb horizontal projection apparatus for projecting images of objects in fluid, etc.

# Journal of Applied Microscopy and Laboratory Methods

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CONDUCTED BY L. B. ELLIOTT.

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# Journal of Applied Microscopy and Laboratory Methods

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NUMBER 2.

## The History of the Microtome.

### I. THE EARLIEST TYPES.

It is proposed to issue a series of articles, of which this is the first, to give the history of the gradual improvement of the microtome. This form of scientific instrument, which has now become so important as to be universally used, is of comparatively recent introduction. The object of the microtome is, of course, to prepare thin sections of soft tissues for microscopical examination. Such an instrument would naturally not have been designed until a demand had arisen from scientific men for the making of sections. Now sections, although used more or less during the early half of this century, were not much relied upon until the second half of the last century had been reached, and we may say that it was approximately about 1860 that section cutting began to come into favor among microscopists. But it was not until 1874 that microtomes began to make their way. I was at that time in Europe and found in the summer of that year that the Ranvier microtome was just gaining its place among the French, being used in Ranvier's laboratory at the College de France, and in a very few other places. Visiting Germany later in that same year, I found that a German modification of Rivet's microtome was being introduced in the laboratories of that country. This modification was known by the name of the Leyser microtome. With both of these primitive instruments I have myself made many tens of thousands of sections. As I have since been constantly occupied with microscopical investigations, I have had an opportunity to follow the gradual development of the instrument, the whole history of which falls within the period of my personal experience as a professional biologist.

We ought perhaps to regard as the earliest form of microtome the instrument which has now fallen entirely into disuse, Valentine's double knife (Fig. 1). To all the oldest workers this instrument must be familiar, but there are probably many of the younger generation who have never seen one of them. This knife was practically a two-bladed scalpel, the two blades being parallel to one another and fastened to a single handle. By means of a screw and spring the blades could be brought nearer together or separated more widely. The two parallel blades were plunged or drawn through fresh tissue so as to cut off between them what we

should call a section. According to our present standard, such a section would be very clumsy and thick, and indeed our students are now so accustomed to the perfect work of the microtome that most of them probably would scoff at such sections as can be obtained with Valentine's knife. Nevertheless, it was an instrument of great service and was employed successfully in the investigations, which laid the foundations of modern histology.



FIG. 1.—Valentine's Double Knife.

There followed after this a series of devices for the obtaining of sections. It seems hardly worth while to attempt to look up all of these, for they are hardly more than matters of curiosity. It will suffice to refer to Prof. Victor Hensen's "Querschnitt," which was described in Vol. II of the *Archiv für Mikroskopische Anatomie*. This instrument was designed for making the sections under the microscope and submit them to immediate examination in a fresh state. Mention may also be made of the instruments designed respectively by Oschatz and Welcker. None of them were of a sufficiently practical character to come into general use.

The first microtome of which I have any knowledge, and which corresponds at all in principle to those now in use, is that which was designed by Prof. W. His, and described by him in 1870 in the *Archiv für Mikroskopische Anatomie*, Vol. VI, page 229. This instrument seems to me

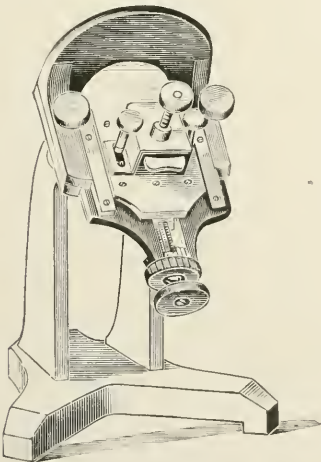


FIG. 2.—His's Microtome.

worthy of attention, and I therefore give the figure (Fig. 2) and brief description of it. Its most essential parts are the object holder, moved in a fixed plane by means of the micrometer screw, and a plate of metal at right angles to the plane of the object holder to guide the knife. As will be seen by the illustration, the instrument was mounted upon a little stand and was placed at an inclination, so that in the actual cutting the section made lies somewhat on the blade of the knife. The object rested on the plate so that the edge of the knife passed through the object down to the plate itself, an operation which, of course, was very likely to injure the edge of the knife. But the knife itself was guided and the object was moved mechanically,

so that in this instrument of Prof. His's we find already two essential conditions, which must govern every microtome, a mechanical precision in the movement of the knife and the mechanical regulation of the "feeding" of the object to be cut. All that subsequent microtomes have accomplished has been to attain these two objects more perfectly, to make the instrument more convenient, and to add such devices as make it possible to work it automatically.

I think it may be not without interest to quote from Prof. His, for nothing could better illustrate how much we have progressed since 1870 than his own statement of the advantages of his instrument. He says, "I have used the instrument since 1866 and have prepared during this epoch over 5000 sections, (5000 sections in four years! Students often prepare as many now in a single day). Without this apparatus I might have been able, like others undoubtedly, to prepare many a fine section, but certainly not such a large number. I have thus gained in time and also saved material; but what is more important than this, and in itself a very important gain, I consider to be the fact that this instrument has permitted a precision of work which would never have been possible with hand sections. It has rendered it possible for me to obtain uninterrupted series of sections of the object to be investigated. To obtain plastic conceptions from pictures of sections is unquestionably a round-about and fatiguing way to the end desired, but we cannot escape it. The objects are too small for us to observe their plastic form directly. Everyone may easily experience how important the uninterrupted series of sections may be for such reconstructions, or plastic views, if he will take the trouble to reproduce his formed conceptions in wax or clay. Thus the new microtome has become entirely indispensable to me in embryological investigations. Another object for which it has shown itself very useful is the retina, and I may call attention to the fact that with the help of this apparatus one may determine very exactly the typography of the yellow spot. Perhaps it may also show itself very useful in many an investigation of small animals. On the other hand, I should not estimate its importance for the investigation of large organs so highly."

This instrument was manufactured by the *Société Genevoise pour la construction d'instruments de physique*. Those who are sufficiently acquainted with the history of embryology to appreciate the great value of Prof. His's monograph on the Development of the Chick, and of his many other papers, will be quick to recognize that this microtome proved its value by the results which it helped to secure. Prof. His's microtome, however, did not come into very general use. This was perhaps chiefly due to the introduction of another microtome by Prof. Leon Ranvier, a microtome on the same general principle, but much simpler in construction and more convenient in use.

The device in question was, so far as I am aware, first described in the French edition of Frey's Histology, prepared by Ranvier, page 712, and was afterwards described and figured in the first edition of Ranvier's *Traité Technique D'Histologie*, page 49. This microtome consists of a tube of brass with a flange-like plate at its top and a micrometer screw at the bottom (Fig. 3). The plate serves to guide the knife, and the object to be cut is placed in the tube and is raised by turning the microtome screw. In Ranvier's laboratory this little apparatus was much used. The practice was to embed the object to be cut in elderrpith, which was then pressed into the tube of the microtome; the pith when moistened with alcohol swells a little; and if the operation is carried out with care and skill the object will be held sufficiently firm for cutting purposes. The instrument



FIG. 3.  
Ranvier's  
Microtome.

used for cutting was the ordinary razor. By a natural assumption,—the razor being the instrument with the sharpest edge then in use,—it was supposed that a knife in the form of a razor would be the best for use with the microtome. How utterly erroneous this notion is I shall have occasion to explain later. In order to work with objects of different sizes and to be able to have several embedded at once, Ranvier added to his microtome two or three tubes which could be placed within it, one inside of the other, but this addition of extra devices was not of much practical value. It will be observed that the essential principles of Ranvier's microtome are the same as those of His. The knife is moved by the hand, but is guided by being rested against the plate. The object to be cut is raised by the screw. But whereas in His's microtome the knife edge came down against the plate, in Ranvier's it passed freely across the object, and in this way escaped the danger of injury which was inherent in His's construction.

CHARLES SEDGEWICK MINOT.

Harvard Medical School.

### On the Manipulation of Sections of Leaf Cuticle.\*

The cuticularization of the outer epidermal cell-wall is one of the most strikingly variable structural features presented by the higher plants as a means of adaptation of a species to the various climatic conditions which control transpiration. Not only do many species show evidence of having thus become adapted to extreme humidity or aridity, for example, through generations of exposure, but, as has been shown by the investigations of Kohl, Lothelier, and others, individuals of the same species manifest a great capacity to vary in this respect. I have recently made a very extended histological investigation of the foliar cuticle of several species, and have gained some experience in the manipulation of sections, which may be of some interest, although I have nothing essentially new to offer. Having somewhat simplified the methods in common use, I offer an account of my experience for what it may be worth.

In the study of a thin cuticle, as a rule, very thin sections are required, two micromillimeters, or even thinner, being usually found necessary. It would, no doubt, greatly facilitate matters in collecting material simply to remove at once a small strip of epidermis freed from the underlying veins. It is no easy matter to keep a blade sharp enough to cut sections of such thinness, and the lignified portions of the leaf have a distinct tendency to dull the edge. I found, for instance, that it is much easier to keep the knife sharp when cutting through a leaf grown in a saturated atmosphere than one of the same species grown under normal conditions. To facilitate handling the object in the process of embedding and trimming, it would, no doubt, be desirable to stain *in toto* with some easily removable stain before embedding.

In my own experience, I have taken a very narrow strip of the leaf and embedded in paraffine according to the usual process. The paraffine is trimmed away under the lens until the surface to be cut is reduced to a minimum. The blade of the microtome knife is adjusted in the slanting position, and the sec-

\* Read before Section G, A. A. A. S., Washington meeting, Dec. 30, 1902.



tions cut off in scrolls. The scrolls are placed on a small drop of distilled water, previously placed in the center of a well-cleansed glass slip. Here they usually flatten out of their own accord, if the knife has been well sharpened and the paraffine is of the proper hardness. Especially obstreperous scrolls are flattened by holding sufficiently high above a small flame not to allow the paraffine to melt. Usually, however, in the case under consideration, it is found more desirable to cut a few extra sections and place them on the drop of water, selecting for study only the ones that unroll of their own accord.

The slide is then set aside under a loosely placed bell-jar, and the water allowed to evaporate spontaneously. After it appears dry the slide is held over a low flame until the paraffine just begins to melt. It may then be stored away and kept indefinitely in that condition.

The remainder of the process is similar to the one usually followed, the paraffine being dissolved off with xylol, the slide carried through alcohol and even washed under a stream of water without disturbing the sections, which are held to the glass by simple adhesion. I have within the past two years thus handled hundreds of leaf sections of several species and do not recall a single instance where a properly cut and flattened section came off in the usual process of staining and mounting.

The process here outlined is a slight modification of Nussbaum's (Anat. Anz., Bd. XII, 1896, pp. 52-54; ref. in Zeits. wiss. Mikr., XIII, p. 309). I am not prepared to state from experience how far it is applicable to the treatment of other plant tissues.

Of all the stains I have tried for the cuticle, Sudan III, proposed by Buscaglioni (Sep. from Malpighia, XII, p. 1-20), has proved most satisfactory for permanent mounts. A double stain with the latter and Delafield's hæmatoxylin succeeds very well for photomicrographic purposes.

My practice is to treat sections with alcoholic solution of Sudan III until the cuticle has taken on an intense coloration, pass rapidly through alcohol to water, thence into dilute hæmatoxylin, where they remain until the cellulose walls assume the desired intensity of color. The sections must be finally enclosed in glycerine or glycerine jelly.

SAMUEL M. BAIN.

University of Tennessee.

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ARTHUR L. DEAN, in an article on Experimental Studies on Inulase, gives the following formula for the medium on which pure stock cultures of *Aspergillus niger* and *Penicillium glaucum* were kept:

Agar-agar, 10 gms.; NaCl, 2.5 gms.; peptone (Grübler's), 2.5 gms.; beef extract, 1 gm.; inulin (air dry), 5 gms.; water, 500 c. c. The inulin used was prepared from *Dahlia variabilis* by alcoholic precipitation.—Bot. Gaz. XXXV, 1.

## A New Changing Nosepiece.

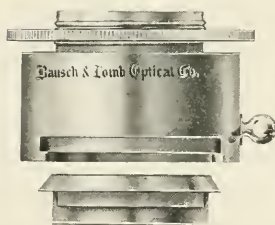
So many different styles of nosepieces, objective changers, etc., have been devised that it may appear superfluous to add another to the list.

The nosepiece illustrated has, however, advantages which, I believe, are sufficient to warrant its finding a place among practical laboratory appliances.

For petrographic, metallographic and photographic work a readily changeable nosepiece which will not interfere with the manipulation of the specimen or the mechanical stage is desirable.

This form of nosepiece has been produced in a number of varieties, with portions of the thread cut away, in the form of a lathe chuck, with spring fork to clutch a ring screwed to the objective, and the sliding wedge form objective changer.

In the first two forms the objective cannot be placed in position without raising the body tube, except when changing low power objectives. In the third form there is little security and the objectives are not liable to center. In the last the only real objections are the expensive construction, the weight, and the need of a special box to contain the objectives fitted with the slide pieces.



Changing Nosepiece.

The nosepiece illustrated consists of a circular case, one end provided with the society screw thread for attaching to a microscope (a clamping ring is fitted to the thread so that the nosepiece may be brought to the proper position), and at the lower end the case is cut away at one side to permit the passage of the rings carrying objectives. These rings are designed in a manner not only to admit of ease in placing them in position, but also to ensure centering of objectives.

The lower surface is flat, whereby the rings can be easily placed or displaced. The upper portion is conical on the outside, and fitting, as it does, the inside adjustable bearing of the nosepiece, the ring is brought to a fixed center.

The adjustable bearing is inside the case and is forced down upon the ring by means of a stiff spring. The raising of this bearing is accomplished by a lever, in an inclined slot, ending in a knob on the outside of the case; this imparts a rotary motion to the adjustable piece and is superior to a straight up and down movement.

All parts of the nosepiece must be carefully made and adjusted, and it is presumed that the objectives have been centered and made parfocal with each other; otherwise good results cannot be expected.

The objectives can be placed in the optical axis by pressing the small lever to one side and sliding the objective, with ring attached, into the clutch; upon releasing the lever the objective is centered by the conical objective part being forced into the conical rim of the nosepiece by the spring of the inside bearing.

Objectives made parfocal will remain so when used on this nosepiece, and the objectives can be changed without raising the body.

The nosepiece is light and small, and not at all in the way when manipulating the specimen. The rings are so small that objectives can be kept in the boxes regularly supplied with them.

W. L. PATTERSON.

## A Simple Method for the Preparation of Auerbach's Plexus.

The methods of preparation of Auerbach's plexus ordinarily recommended, involving the use of osmic acid and other expensive agents, have made the demonstration of this interesting structure unavailable to many workers.

Auerbach's plexus has been easily and cheaply prepared in our laboratory course in Histology by the following method, which may be not without interest and value to general workers.

After the intestine has been macerated (Stohr, '00, Histology, p. 272) the muscularis can be easily peeled off in long strips, often half an inch wide. The strips are now pinned onto a cork or piece of wood in water, Fig. 1, care being taken in drawing the tissue smooth not to stretch it at all. The mounting of the tissue on the cork has two advantages; first, that it cannot wrinkle while being treated by the various re-agents; second, that the minimum amount of fixing and staining re-agents is used as the cork can simply be inverted in a suitable dish containing the desired fluids.

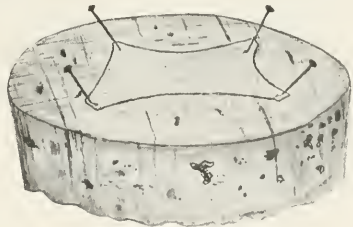


FIG. 1.

After the tissue has been placed on the piece of wood or cork, it is ready to be fixed. The ordinary re-agents may be used, but those containing osmic acid seem to give the most satisfactory results. The best results in staining were secured by the use of Heidenhain's hæmatoxylin and Brazilin, the first giving the better differentiation.

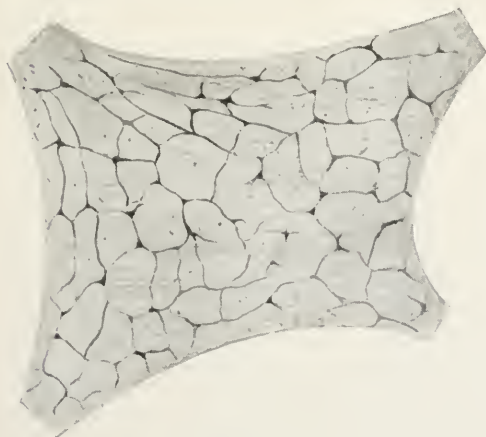


FIG. 2.

The following method was employed:

Hermann's Fluid, 5 min.

Wash in running water 1 min.

Iron solution, 2 per cent., 15 to 30 min.

Wash in running water 1 min.

Heidenhain's hæmatoxylin, 15 min. to 1 hour.

Wash in running water 1 min.

Iron solution until excess of stain is removed, 3-10 min.

The tissue is now removed from the cork to a glass slide or a watch glass, where the de-staining may be watched. For this purpose

it is sufficient to place the slide over white paper, or on the microscope. When the differentiation has been completed by this further use of the iron solution on the tissue while it is on the slide, one can see the plexus without any mag-

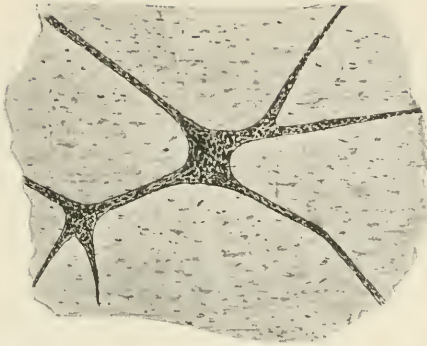


FIG. 3.

A. J. May from preparations made by the method described above.

Meissner's plexus can be prepared by the same method.

Zoölogical Laboratory, Syracuse University.

nification, and it can be washed in water, and is now ready to be mounted in glycerine jelly by the usual process. When once one has a large piece stained and in glycerine, it may be cut into pieces of a suitable size to mount. One can frequently get a dozen preparations from one large piece.

This simple process gives very beautiful results and avoids the use of expensive re-agents and is not difficult to manipulate.

Figures 2 and 3 were drawn by Mr.

W. M. SMALLWOOD.

## On the Occurrence of Parasites in the Blood of the *Damonia Revesii* Turtle.

Since Laveran has shown that malarial fever is caused by the presence of parasites in the blood, many investigations covering this subject have been made, and in consequence the life history of the malarial parasites has now been pretty thoroughly investigated, and their morphology fairly well understood.

There is, however, another class of hæmatozoon, belonging to the hæmosporidia family, which invades the blood of birds, reptiles and batrachians, of which not so much is known, though likewise of much interest, and it is upon this subject that I propose to say a few words to-night.

The generic name of hæmosporidia, which I have used in this article, was proposed by Celli and Sanfêlice and adopted by Krusé.

The hæmosporidia are corpuscular parasites, and during ochizogonia inhabit the red blood cells, and only occasionally are they found in other parts of the body.

In the case of birds and mammals, sporogonia of the better known hæmosporidia takes place extra-corporeally, in the intestine of the mosquito. It is also probable that in the case of cold-blooded animals there is no change of host, and that sporogonia takes place in the blood cells of the infected animal.

The hæmosporidia bear a near relationship to the coccidia, and in consequence might be designated as the blood-infecting coccidia, and in order to distinguish them from the true coccidia, which invade certain organs of the body, and not the blood.

In its youngest stage the blood-infecting hæmosporidium appears as a very small unicellular amœboid body. In growing they change their shape, which may more or less conform to the contour of the invaded blood cells.



In addition to the amœboid motility, the hæmosporidia are possessed of a more general characteristic, namely the formation and deposition of a dark-colored pigment, which, in the growing parasite, is formed at the expense of the hæmoglobin of the red blood corpuscles. The nuclei of the parasites, which may be located in the various parts of the body, analogously to the coccidia, are provided with karyosomes.

In an article entitled "On the Hæmosporidia in American Reptiles and Batrachians," by Dr. Gustav Langmann (studies from the Department of Pathology, of the College of Physicians and Surgeons, Vol. VI, p. 29), an account is given of several parasites which were found in the blood of frogs, snakes, and turtles, and as they bear a striking resemblance to those found by me in the blood of the Chinese turtle (the *Damonia revesii*), I thought that a short description of the same would not be out of place.

My investigation upon this subject began in the latter part of December, 1901, at which time I purchased a small *Damonia revesii* turtle from a bird fancier.

A few blood smears were made from the tail-blood of the animal, and which were stained with Goldhorn's polychrome methylene blue and eosin. A careful microscopic examination of these smears soon established the fact that no parasites were present.

The turtle was then laid aside and nothing more thought of the matter, until about three months later, when curiosity again prompted me to re-examine the blood of this turtle.

Fresh smears were, therefore, made and stained by the Goldhorn reversed method, and upon examination were, much to my surprise, found to be infected with both young and fully matured parasites of the Hæmosporidia family.

Two kinds of parasites were noticed; one form being long and slender, with usually no noticeable nuclei; the other being large, stout, bean-shaped, and most always nucleated. The latter parasite seemed to preponderate over the former.

It will be seen from the accompanying photomicrograph that the parasite of type 1 is long and slender; either straight or slightly curved. The nuclei of the invaded cells will be found to have been pushed aside laterally to make room for the parasites, which occupy a large portion of the red blood corpuscles. (See plate 4.)

I am at present unable to say anything further concerning the morphology of type 1 parasite, as it was only observed in the form first described.

#### TYPE 2 PARASITE.

In its youngest form this parasite is a small spherical nucleated body located near the periphery of the invaded blood cell. (Plate 2, Fig. 1; Pl. 3, Fig. 1.)

In growing, the parasite gradually lengthens out, assuming more of an ellipsoid form, with the nucleus located near the periphery of the parasite. At the same time it will be noticed that the corpuscular nucleus is slightly moved toward one end of the red corpuscle. In some cases there may be noticed an enlargement of the red cell nucleus. (Plate 2, Figs. 2, 3, and 4.)

We may also occasionally find a red blood cell doubly infected with this young form of parasite when the cell nucleus will be found to have been shoved down nearly to one end of the corpuscle. (Pl. 3, Fig. 2.)

Langmann, I believe, records several cases of multiple infection of the mature parasite, but is silent upon the multiple infection by young parasites.

In continuing their growth the ellipsoid form of the young parasite becomes still further elongated and assumes the form of a crescent or bean. Their nuclei will be found in various parts of the body, though usually they are located near the center.

Even at this early stage of the parasite's development the body will be seen to contain granulations and other characteristic markings. (Pl. 2, Figs. 5, 6 and 7. Pl. 1, Fig. 9. Pl. 3, Figs. 4 and 5.)

From this stage of its development until fully mature, the parasite undergoes no material change of form, except that it increases in size, and may eventually



PLATE I.



PLATE II.

become so large as to practically fill the blood corpuscles, and at the same time crowd the nuclei nearer and nearer towards one end of the cells. (Pl. 1, Figs. 2, 3, 4, 5, 6, 7, 8 and 10. Pl. 2, Figs. 8, 9, 10, 12; also Figs. 11 and 13. Pl. 3, Fig. 5, which shows the parasite in its extra-corpuscular form.)

The parasite shown in Pl. 2, Fig. 11, differs in some respects from the general run of parasites observed by me in the blood of the *Damonia revesii* turtle. It will be observed that in this particular parasite the chromatin of the nucleus is stretched out lengthwise, nearly extending from one end of the parasite to the other, whereas usually the parasitic nucleus is round or ovoid in form.

In Pl. 3, Fig. 5, we also find another deviation from the regular order of things. The parasite in question was noticed to be surrounded by a faint colorless zone, or halo, which suggested the possibility that the parasites are surrounded by a capsule. As this, however, was the only parasite out of a great many, which was surrounded by a colorless zone, I am at present unable to throw more light upon the subject.

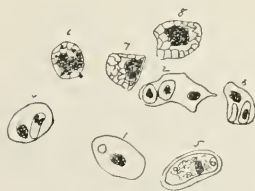


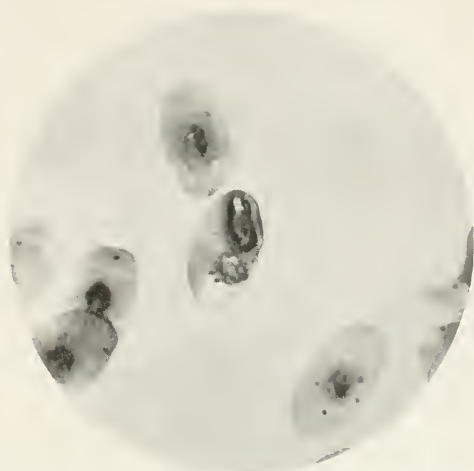
PLATE III.

Referring again to the growth of the parasites, it will be observed that as they increase in size, the markings in the body or cytoplasm of the parasites become more and more accentuated and eventually, in the fully mature parasite, present the appearance of a coarse granulation or reticulum. (See photomicrographs.)

Sometimes the parasites grow to be so large that they cannot accommodate themselves to the size of the blood corpuscles, in which event one end of the parasite may be considerably bent over (Pl. 1, Fig. 1), and may even, according to Langmann's observations, be bent over so far as to form a loop, or bight, but personally I have not seen any that were disfigured to this extent.

Langmann has also recorded the direct invasion of the corpuscular nuclei by the parasites, but, though I have looked long and hard for such an occurrence, I have failed to observe it in the blood under examination.

PLATE IV.—Parasite in blood of *Damonias revesii* turtle,  $\times 1000$ . Red cell infected by young parasite.

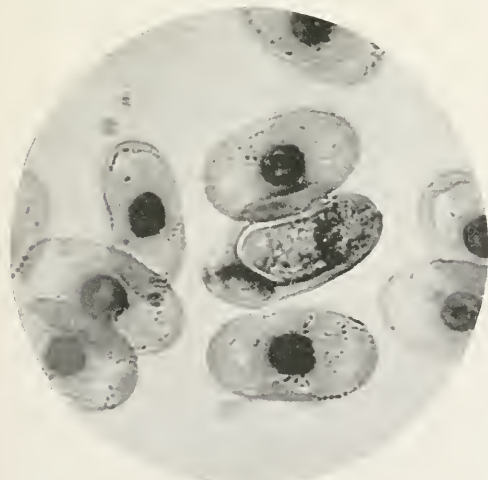


About three weeks ago I bought another turtle of the same species as the first one, and upon examination its blood was found to be free from parasites. It was placed in the same aquarium with the first one, and about two weeks later its blood was examined again, and this time it was found to be infected with the same parasite as that observed in No. 1 turtle, excepting that in this case the majority of the parasites were found to be in the young stage of development.

I have tried to construct the life cycle of these parasites, but, up to the present time, I have not been successful, but hope, later on, to have more information upon this subject.

The average size of the mature parasite was found as follows:

PLATE V.—Large living parasite in red blood cell of *Damonias revesii* turtle,  $\times 1383$ . Shows marked granulation of parasite cytoplasm and shows the red cell nucleus down in one corner.



Length,  $9.4 \mu$ ; diameter,  $4.4 \mu$ ; minimum length,  $7.8 \mu$ ; minimum diameter,  $2.8 \mu$ ; maximum diameter,  $6 \mu$ .

The parasite bears a striking resemblance to the *Hæmoproteus danileus* Ki, but as this parasite only invades the blood of birds, it cannot, therefore, be the same as that found by me in the blood of the *Damonia revesii* turtle.

Langmann thinks that the parasites he found in the turtle blood are the *Drepanidium ranarum*, and my Type 2 parasite may be the same as those found by him, but, until I am better informed upon this subject, I prefer to simply designate them as the parasites of the *Damonia revesii* turtle.

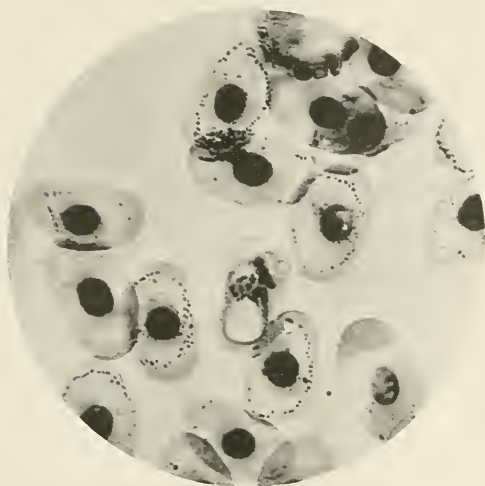


PLATE VI.—Blood of *Damonia revesii* turtle showing infection of corpuscle by large mature parasite, x 1000.

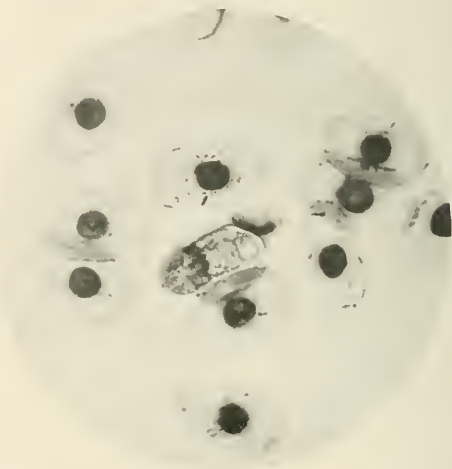


PLATE VII.—Blood of *Damonia revesii* turtle showing the infection of a red cell by a large mature parasite. Possibly *Drepanidium ranarum*.

In conclusion I would say that since the purchase of my second turtle I have added to my collection 1 small tortoise, 1 mud turtle, 1 snapping turtle, 1 Southern soft-shelled turtle, and 1 Southern green turtle, all of which were examined when first purchased and found to be free from parasites, and although they have now been in daily contact with the two infected Chinese turtles, none of them so far has shown any sign of infection, but what may be the final result remains to be seen.

JAMES H. STEBBINS, JR.

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THE University of Michigan has secured the lease of a piece of ground containing about seven acres for a botanical garden and arboretum. The land is located in immediate proximity to the campus and adjacent to other university property. It includes a "kettle hole," which will be utilized for the growth of aquatics, and has the advantage of great variety of slope, soil, and exposure. The front nearest to the university buildings is reserved for the erection of a conservatory with workrooms.—Bot. Gaz. XXXV, 1.



## The Museum.

### III.

#### THE MUSEUM BUILDING—INTERIOR.

Museums can be broadly grouped, in regard to form, in two classes, (1) the single (sometimes double) storied spreading structures with vertical or top light, more or less combined with side-lights (Metropolitan Museum of Art, New York; Field Museum at Chicago; National Museum, Washington, D. C.; World's Fair buildings everywhere), and (2) the multi-storied, more restricted, upright buildings with side wall windows and lateral light (American Museum of Natural History, New York; Natural History Museum, London, etc.).

The question of the comparative merits of the single or multi-storied buildings, apart from considerations of space and financial resources, probably rests upon two particulars, first, that of wall space, and that of light, the latter with



FIG. 12.—Central Hall (top light) Metropolitan Museum of Art, New York.

especial reference to reflections. Reflections are puzzling and vexatious accidents, and in many instances, under peculiar conditions, destroy the pleasure of visitors completely. To look in a case and find yourself mirrored with exacting fidelity at every step you take, decidedly upsets the spectator's interest and enjoyment in an exhibit. Or in examining objects at an angle to have them replaced by the figures and faces of bystanders is equally disconcerting.

Obviously, the filling of the case with light, so that the emitted light is in excess of the transmitted light, will rectify this defect. And the influx of light from above *has a tendency* to produce just such a necessary contrast. But it does not dissipate reflections entirely, and on dark days such misfortunes are about as conspicuous in skylighted buildings as in those with windows. It is an unavoidable optical effect which can be reduced, never, when conditions pro-

voke it, be dissipated. Dark interior objects, as black or brown animals, dark backgrounds, etc., usually suffer from this interference. The observer can himself largely diminish this effect by bringing his eyes close to the glass.

All this, of course, applies only to glass cases; free objects are exempt from this disparagement. Art exhibits, as sculpture, architectural models, etc. (Fig. 12), probably are better shown in a vertical light, though this conclusion is not unquestioned, as in high halls with high windows this species of exhibits appear also to advantage. Overshadowing advantages must be clearly shown in favor of vertical light to overbalance the oppression produced by sealed rooms. In the case of small halls the sense of imprisonment they produce is most dismal.



FIG. 13.—Hall of Metropolitan Museum of Art, New York, showing very adequate illumination furnished by lateral windows.

But the single story skylighted building offers undoubted advantages in continuous wall spaces. It certainly seems to furnish some economy in that direction. But this claim is hardly substantiated, as pier cases, projecting inward to the interior of the hall, multiply wall spaces effectively and generally more than replace the wall space sacrificed by the introduction of windows.

If continuous wall spaces are desired there need not be a resort to skylights, as lateral windows, at a height of ten feet from the floor, extending to the ceiling, furnish very adequate illumination (Fig. 13). Such an arrangement obtains in the Metropolitan Museum of Art in New York, and in the case of large, coarse objects, as ancient pottery, glass, sculptures, etc., meets every need. But an illumination of this sort, either for very small objects placed on shelves along the walls, or in flat cases in the hall, is not the best. Pier cases between windows produced to the floor secure a more useful disposition of light, while the completed window, flooding the room with light from both sides of the hall, will

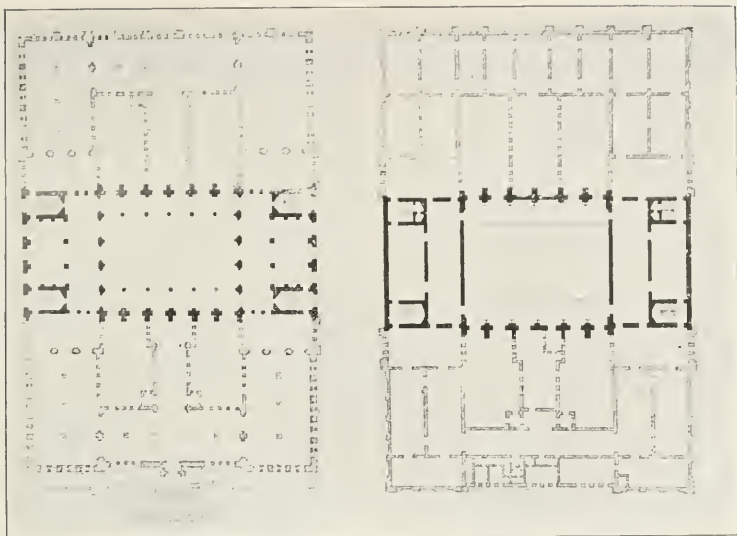


FIG. 14.—Ground Plan, Metropolitan Museum of Art.

prove sufficient for flat cases also. As regards reflections, it seems incontestably to be true that in window lighted halls they are less obvious in north and south buildings than in east and west buildings, since in the former there is less disparity in the amount of light from the two sides of the hall than in the latter, that is, in the average throughout the year.

The general advice can be hazarded that single or double storied skylight or clear story buildings should be designed for art museums, and many storied

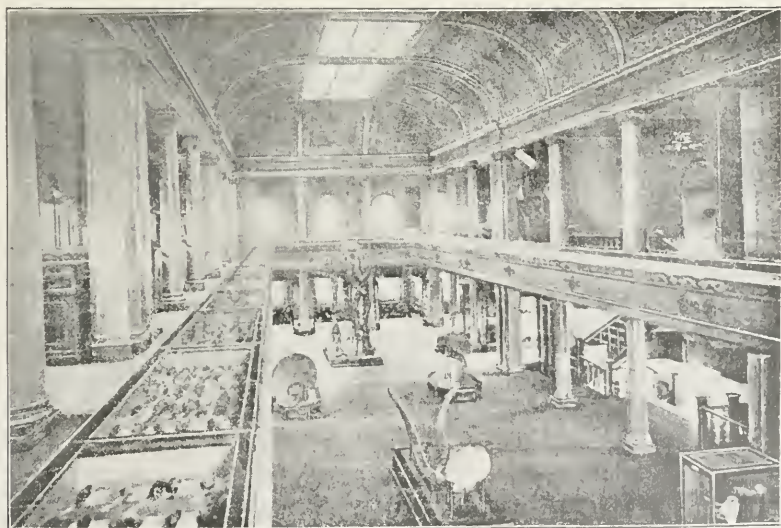


FIG. 15.—Hall of the Chicago Academy of Sciences.



side lighted buildings for science museums, while a moderate interjection of the features of each into the other is permissible, perhaps in some cases desirable. (Some of the subordinate halls in the Natural History Museum in London are abundantly filled with with light from top and side lights.)

For illustration, the central dome-covered apartment of the Metropolitan Art Museum is surrounded by courts, halls and exhibition spaces lighted from the sides (Fig. 14), and the top floors of science museums admit of a skylight treatment as in the second floor and gallery of the Chicago Academy of Sciences (Fig. 15), while a more complete assumption of the art form is shown in the University Museum, Oxford, holding the Pitt Rivers collection (Fig. 16), in the main hall of the Natural History Museum, London, and in the Museum of the Jardin des Plantes, Paris, in the Museum of Science and Art, Edinburgh, Zoölogical Section of the Dublin Museum, etc., not, however, it appears to me, with the most admirable results.

A *bastard* effect of vertical lighting is effectively used in science museums



FIG. 16.—University Museum, Oxford (Pitt Rivers Collection).

where a gallery, running around a lower hall and plentifully lighted by windows, allows its light to fall upon the floor below. It is a mistake, as seen in many European museums, to exaggerate the limits of this concession by carrying two stories about a lower hall, in the form of galleries. The effects then become lugubrious (Fig. 17). Such gallery structure is attractively shown in Fig. 1, and, in spite of inartistic installation, not unpleasantly in the Manchester Museum in Owens College, Manchester, England (Fig. 18).

The faulty palisaded dreariness of the superimposed galleries might be helped somewhat by always maintaining a very wide main floor, and successively narrowing the width of the galleries above it.

A word may be written about the entrances of museums. They are important features; I mean the first view inside the museum walls. Visitors should not enter against a blank wall, and be limited in their impressions to a check counter and a turn-stile. Expansion is the emotion to evoke at that moment. (See





FIG. 17.—Hall of Science and Art Museum, Dublin.

chapter on *Effect*.) A room, a hall of large objects, or a vestibule treatment leading to the halls, with a symbolic motive, can be serviceably employed. It is a psychological moment and the summary of impressions, however vague at that instant, should be generally large, suggestive, dignified and inviting. The entrance to the Natural History Museum, London, partakes of some grandeur,



FIG. 18.—Hall of Manchester Museum, Manchester, England.

though the stone pillars and a sort of cathedral cavernousness is a trifle dismaying. Darwin's statue is the first reassuring thing the visitor encounters. The treatment could be enormously improved.

American Museum of Natural History.

L. P. GRATACAP.

## Methods in Plant Physiology.

## VIII.

## CARBON ASSIMILATION.—Continued.

12. **Identification of the Gas Produced in Carbon Assimilation.** The gas which is produced as a by-product in photosynthesis may be easily collected and identified as oxygen, if a sufficient quantity is obtained.

Fill a jar with water to within a few centimeters of the top; select a funnel whose diameter and height are a little less than those of the jar. Into the funnel place a dozen vigorous stems of *Elodea canadensis*, or other water plant, and sink the funnel, bowl downward, into the jar of water, including all of the plant material within the funnel. Fill a test tube with water and, without admitting any air, set it down over the submerged stem of the funnel. Keep the jar in sunlight until the test tube is nearly full of gas, then, without raising the mouth of the tube above the water, detach it from the funnel and cover the open end with the thumb. Test the contents of the tube with a glowing splinter.

13. **The Evolution and Absorption of Gases in Carbon Assimilation.** (a) *Bous-singault's Phosphorus Method.*—Fill a tall bell-jar over water with hydrogen and carbon dioxid, the latter should compose 8 to 10 per cent. of the mixture. It is best to keep the bell-jar and water in a large pan which will admit of being easily transported. Place a small potted plant, *e. g.*, a *Coleus*, under the bell-jar before any gases are admitted. Attach a small piece of phosphorus to a curved wire and introduce it under the bell-jar, allowing it to project above the surface of the water. The oxygen in the intercellular spaces of the plant will unite with the phosphorus, filling the bell-jar with the white fumes of the  $P_2O_3$ . Place the preparation in the dark until the fumes have been absorbed by the water, which will occur in two or three hours; then place the bell-jar in strong light, noticing how quickly the white fumes reappear.

(b) *Pfeffer's Method for Estimating the Quantity of Carbon Dioxid Consumed in Photosynthesis.*—This experiment consists in exposing a leaf to light in an atmosphere containing a known amount of carbon dioxid and actually measuring the amount consumed. The necessary apparatus is shown in Fig. 8; the glass tube is 15 mm. in diameter and about 100 cm. long, the upper end is blown out into a balloon. The open end of the tube dips into a dish of mercury. Attach a fine wire to the petiole of a leaf of *Nerium oleander*, roll the leaf into a cylinder and thrust it up the tube until it reaches the wide part, where it will unfold of itself. The tube is held in position over the mercury by means of a burette holder. A few drops of water are placed above the mercury column in the tube to protect the leaf from vapors of mercury. By means of a curved pipette remove some of the air from the tube, thus causing the mercury to rise a short distance. Record the volume of the air in the calibrated tube, also the height of the little column of water in the tube. Readings of the barometer and thermometer are also taken.

Pass in 8 or 10 c. c. of pure carbon dioxid, which has been freed from hydrochloric acid by washing in sodium bicarbonate solution, then note the volume accurately. The apparatus is now exposed to bright diffused light for six to twenty-four hours. When the exposure is completed the leaf must be pulled out by means of the wire. After the apparatus has returned to room temperature, readings are again taken. By means of a curved pipette introduce 1 or 2 c. c. of a concentrated solution of potassium hydroxid into the tube, using great care not to admit any air. At the end of two hours the surplus of carbon dioxid will be absorbed, and careful readings are again taken. The volume of the leaf must be ascertained and subtracted from the gas volume; it may be found by immersing the leaf in 50 per cent. alcohol, contained in a graduated cylinder. To obtain the absolute volume, it is necessary to reduce the readings (before and after the injection of the potassium hydroxid) to  $0^{\circ}\text{C}$ . and 1 meter mercury pressure and to correct for the tension of water-vapor, etc. This is to be done by the formula of Bunsen,

$$V_1 = \frac{(v-m)(b-b_1-b_2)}{1+0.00366t^{\circ}}$$

where  $v_1$  = reduced volume of the gas,  
 $v$  = the observed volume,  
 $m$  = the correction for the meniscus,  
 $b$  = the barometric reading,  
 $b_1$  = the mercury pressure in the tube,  
 $b_2$  = the water-vapor tension at the temperature  $t^{\circ}$ .

(c) *Engelmann's Bacterial Method.*

—In this method the presence of oxygen is detected by the extreme sensitiveness of certain bacteria to that gas. It is claimed that the presence of the billionth part of a milligram of oxygen can be ascertained by this test.

In order to obtain the bacteria, kill a pea by placing it in boiling water, then allowing it to putrefy in 200 c. c. of water. The best results will be obtained if the bacteria thus procured are transferred to agar cultures.

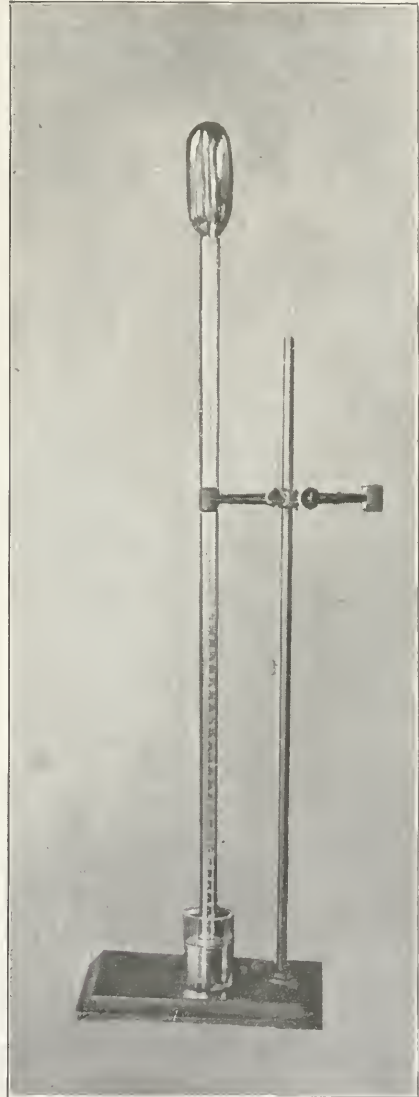


FIG. 8.

Place some bacteria on a slide under a cover-glass; notice how they swarm around air-bubbles and finally collect at the margin of the cover-glass, where oxygen is most available. Make another preparation and seal the edge of the cover-glass with olive oil or vaseline in such a way as to prevent the access of oxygen, notice that the bacteria ultimately become sluggish and come to rest. If a filament of *Spirogyra*, or a bit of a leaf of some water plant be included with the sealed bacteria, oxygen may be produced by exposing the preparation to light. Cover the microscope, upon which the slide rests, with a tight box which will exclude all light. When it is found that the bacteria have come to rest, remove the box and examine immediately to note the return of motility.

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## A Review of the Methods of Staining Blood.

### V.

#### D. *Neutral Stains.*

1. **Acid Fuchsin-Methylen Blue.**—*Ehrlich's* neutral compound of acid fuchsin and methylen blue is made as follows: To 5 volumes of a saturated watery solution of acid fuchsin is added gradually with shaking 1 volume of a strong watery solution of methylen blue, then 5 volumes of distilled water. After some days the fluid is filtered. Methyl green may be substituted for methylen blue in this solution.

Preparations should be stained from 2 to 5 minutes, washed in water very superficially, dried with filter paper, and after air-dry mounted in balsam. Erythrocytes stain red, acidophile granules bright puple, neutrophile granules violet.

*Tamassia* (1894) claims, that by the use of the following compound of acid fuchsin and methylen blue, he has demonstrated the presence of neutrophile leucocytes in the blood of various mammals:

Saturated watery solution of acid fuchsin	5 c. c.
“ “ “ “ methylen blue	1 c. c.
Distilled water - - - - -	5 c. c.

2. **Phloxin-Methylen Blue.** *Rosenberger* (1901) gives this formula for a neutral stain:

Saturated watery solution of methylen blue -	50 c. c.
“ “ “ “ phloxin - -	20 c. c.
Alcohol, 95 per cent. - - - - -	30 c. c.
Distilled water - - - - -	60 c. c.

These are all mixed indiscriminately together and a bluish solution results. A precipitate generally falls and the stain should be shaken before using.

Blood films fixed by dry heat at 115° to 120°C. for 20 minutes or in equal volumes of alcohol and ether are stained 1 to 3 or 4 minutes, washed, dried and mounted in balsam. Nuclei stain various shades of blue, the nuclei of the lymphocytes stain deeply and around their periphery numerous basic granules are often seen. Acidophiles, nuclei light blue, granules bright red, almost brilliant.



Neutrophiles, nucleus takes the stain quite darkly, granules either a dull pinkish color or a bright red, "denoting that they are slightly acidophile." Myelocytes take a faint blue stain. Nuclei of normoblasts take the basic stain. Malarial plasmodium stains bluish green with pigment granules in contrast.

3. **Picric Acid-Rosaniline.**—*Ehrlich*: This dye is insoluble in cold water, soluble in alcohol or glycerin. A glycerin solution gives in 12 to 24 hours fairly satisfactory results. A quicker staining can be obtained by allowing a hot water solution to drop from a filter on the heated dry preparation for several seconds, wash in water, dry and mount. The neutrophile granules stain quite well, the acidophile granules are easily recognized but are not so well stained as by the glycerin solution.

4. **The Triple Stain of Ehrlich and its Modifications.**—*Ehrlich* in 1883 published his first formula for his acid fuchsin-orange G-methyl green neutral compound. This formula was as follows: To 300 c. c. of a mixture of equal parts of a saturated solution of methyl green, acid fuchsin and orange G, add 60 to 90 c. c. of alcohol. This solution is to be employed only after long standing. Nuclei stain greenish, erythrocytes orange, acidophile granules very dark (red) and neutrophile granules violet.

There have been many modifications of Ehrlich's triple stain.

*Biondi* (1888) prepared at room temperature saturated watery solutions of orange G, acid fuchsin and methyl green (100 c. c. of water will dissolve about 15 grams of orange, 60 grams fuchsin and 10 grams of methyl green). The fluid was shaken often and allowed to stand several days, then filtered. They were then mixed, 10 parts of the orange G solution, 12 parts of the acid fuchsin and 3 parts of the methyl green solution. They must be mixed in order given, and the methyl green added drop by drop with constant stirring. This mixture stains dry preparations in a few minutes.

*Heidenhain* (1888) modified Biondi's solution. 100 c. c. of orange G is mixed with 20 c. c. of acid fuchsin, and 50 c. c. of methyl green added with constant stirring.

The Biondi-Heidenhain triple stain can be obtained prepared in the powder at dealers in stains.

*Bergonzini* (1890) used a mixture of a basic dye (methyl green) and two acid dyes (Wiegert's acid fuchsin and gold orange after Griesbach). 20 grams of each of these dyes were dissolved in 100 grams of distilled water. One part of the red solution, two parts of the green solution and two parts of the yellow solution were mixed, then filtered through cotton. The resulting dark brown fluid remained good many months.

*Aronson* and *Philipp* (1892) used the following modification of Ehrlich's triple stain for the study of leucocytes in sputum:

Saturated aqueous solution of orange G (extra)	-	55
" " " " acid rubrin	-	50
Distilled water	-	100
Alcohol	-	50

To this mixture is then added,

Saturated aqueous solution of methyl green	-	65
Distilled water	- - - - -	50
Alcohol	- - - - -	12

This mixture must then stand 1 to 2 weeks before use. It gives good staining after dry heat or alcohol and ether fixation. Stain 10 to 15 minutes, wash in water, dry in the air and mount. Nuclei are stained green, hemoglobin orange, acidophile granules yellowish red, neutrophile granules violet red.

*Neuser* (1894) used a modified triple stain for staining his "perinuclear basophile granules":

Saturated watery solution of acid fuchsin	-	50 c. c.
" " " " orange G	- -	70 c. c.
" " " " methyl green	-	80 c. c.
Distilled water	- - - - -	150 c. c.
Absolute alcohol	- - - - -	80 c. c.
Glycerin	- - - - -	20 c. c.

Dry preparations stained with this compound show in certain diseases (gout, leukæmia, etc.,) a grouping of dark blue stained granules around the nuclei of the mononuclear leucocytes and over and around the nuclei of the polymorphonuclear leucocytes.

*Prince* (1898) substituted toluidin blue for methyl green and eosin for orange G in Ehrlich's tricolor stain.

Saturated solution of toluidin blue (Grübler)	-	24
" " " acid fuchsin	- - -	1
2 per cent. eosin	- - - - -	2

The solutions should be made in distilled water and should be mixed in the order given and then be thoroughly agitated for a few moments to prevent precipitation of the toluidin blue by the acid dye. Only the supernatant fluid is to be used for staining. This mixture is ready for immediate use after agitation. Stain from 30 to 60 seconds with fresh stain. It possesses good staining qualities at the end of 10 to 12 weeks, but it then requires 5 to 7 minutes' action to produce good results. Preparations should be fixed by dry heat 120°C. for at least 20 minutes.

*Ehrlich* has recommended several modifications of his original formula, the latest (1898) is as follows:

Saturated watery solution of orange G	-	13-14 c. c.
" " " " acid fuchsin	- - -	6-7 c. c.
Distilled water	- - - - -	15 c. c.
Alcohol	- - - - -	10 c. c.
Saturated watery solution of methyl green	-	12.5 c. c.
Alcohol	- - - - -	10 c. c.
Glycerin	- - - - -	10 c. c.

These are to be measured in the order given in the same measuring glass, and on the addition of the methyl green the solution is thoroughly shaken. The solution can be used immediately and remains good for a long time. Only slight fixation is necessary. Stain not more than 5 minutes. Nuclei stain greenish, red corpuscles orange, acidophile granules copper color, neutrophile granules violet.

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## The Technique of Biological Projection and Anesthesia of Animals.

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### XI. THE ANESTHESIA OF ANIMALS.—Continued.

**VERMES: *Leeches*.**—For studying the anatomy of leeches in the live state small specimens showing the greatest degree of transparency should be selected and placed in water, five parts, with one per cent. chloretone solution, two parts. The animals soon become motionless and lose their power of adhesion to the dish, and should then be mounted in a compressor, with enough of the anesthetizing solution to surround them. A gravity compressor, as illustrated and described in the directions for the study of earthworms, is useful for leeches. Specimens bearing ova or young attached to the ventral surface are best studied in a watch-glass, with just enough solution to cover them. Young leeches, when treated as above described, soon release their hold on the adult and lie quietly in the watch-glass. If sufficient water is soon added to dilute the chloretone solution to one in five, or weaker, the little leeches revive, find their mother, and reattach themselves. In species having a protrusible proboscis, this organ is often freely extended under the influence of chloretone, and they may be killed in this condition by the addition of a suitable killing agent after complete anesthesia has been induced.

***Rotifers*.**—Chloretone is a valuable adjunct to the compound microscope in the study of these interesting and, in many species, active animals. To study the action of their cilia, the mastax, and anatomy, mount either free-swimming or attached species in a hollow-ground slide, in a watch-glass or on a plane slide, and add one per cent. chloretone solution, drop by drop, while observations are being made. Ciliary action continues after complete somatic anesthesia has been induced and, by grading the amount of chloretone properly, any desired rate of ciliary activity between normal and complete quiet may be had.

**ARTHROPODA.**—The value of chloretone in studies of animals of this type is at once evident, when we consider that reduced rates of motion of their appendages, and the possibility of placing the live animals in the best position for study with hand lens, and dissecting or compound microscopes, are conditions of success not so easily attained by any other method. Moreover, the action of the anesthetic is such that the movements of the mouth parts are unusually well displayed in many species. The method is limited, as yet, in its application to aquatic species and to others which live in moist places and may be immersed in chloretone solution without drowning.

***Crayfish*.**—Adult crayfish should be placed in full strength, *i. e.*, one per cent., chloretone solution, sufficient to cover them, and allowed to remain for twenty minutes, or until anesthesia is sufficiently complete for the desired studies. Young crayfish, just out of the egg, require a solution of water, five parts, one per cent. chloretone solution, one part. The most interesting and instructive stage for the study of crayfish is during the three or four days fol-

lowing the first moult, or from about the third to the eighth day after hatching. At this age the exoskeleton is so thin and transparent that the following instructive observations may be made: The movement of the gill-paddle, the pulsation of the heart, its strands of muscle fibers, the points of attachment of the heart to the dorsal wall of the body, the valves of the heart and the entrance of the blood during diastole, and its movement into the arteries at systole, arterial and cœlomatic circulation of the blood, muscles of the legs, the stomach, ventral nerve chain and its ganglia in the abdominal somites, and in some animals the gills and other structures. Place the specimens in a watch-glass, with water, five parts, one per cent. solution of chloretone, one part, or more, if needed. For studying the heart, stomach and other organs in the thicker parts of the



FIG. 6.—A Native Flea (*Daphnia*) anesthetized with chloretone and photographed from the screen while the class was studying the pulsations of its heart.

cephalo-thorax, reflect direct sunlight from the mirror of the microscope through a small aperture in the diaphragm, or through a hole in a card placed on the stage of the microscope under the watch-glass. The beam of sunlight should be of less diameter than the width of the carapace of the animal, otherwise lateral rays may temporarily blind the eye of the observer.

*Daphnia*.—This may be taken as an example of the water-fleas. Place the specimens in a watch-glass, or in a hollow-ground slide, with water, five parts, and one per cent. chloretone, one or two parts. The animals become anesthetized quickly, and the degree of anesthesia may be controlled by adding water or chloretone with a pipette. *Daphnia* is interesting from the fact that its heart has an extremely rapid rate of pulsation, but this is reduced by short treatment in strong or long continued immersion in weak chloretone solutions.



The accompanying engraving is from a photograph of a live anesthetized *Daphnia* of medium size, as it appeared when projected on a screen and a class was studying the pulsations of its heart. Only partial anesthesia had been induced by placing the animal in a mixture of six parts water to one of one per cent. chloretone solution, in order that slow normal movements of the appendages might be studied. A slight movement of the antennæ occurred during the twenty-five seconds exposure of the photographic plate. Sunlight and a three-quarter inch objective (Leitz No. 3) were used in the manner described in May and June (1902) issues of this JOURNAL in projecting the image of the live *Daphnia* on the screen. The photograph was made with a Unar lens (B. & L. Opt. Co.), on Cramer's isochromatic instantaneous plate.

*Cyclops and Gammarus*.— These and many other species are to be treated in essentially the same manner as *Daphnia*, the only difference being in the proportion of chloretone to water, and this is readily determined by experiment.

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## The Bacterial Flora of Freshly Drawn Milk.

### IV.

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M. CUMMING.

## Elementary Medical Micro-Technique for Physicians and Others Interested in the Microscope.

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### XIII.

#### SIMPLE PATHOLOGICAL TECHNIQUE.—Continued.

*Embedding in Celloidin.* First prepare two solutions of celloidin. one thin, the other very thick, by dissolving the celloidin in equal parts of absolute alcohol and ether to the proper consistency. It takes a day or so for the celloidin to dissolve. Transfer the specimen from absolute alcohol to equal parts of absolute alcohol and ether for four hours, after which put it in a thin syrupy solution of celloidin for twelve hours, then into thick celloidin for twelve hours. It is now ready to embed. Prepare a small square block of white pine  $\frac{3}{4}$ " square and  $\frac{1}{2}$ " thick as free from pitch and resin as possible and pour a large drop of the thick celloidin on one of its surfaces, which should be roughened. Remove the specimen and embed it in this drop of celloidin. Pour additional celloidin over the speci-



FIG. XXV.—Epithelioma of the hand showing nest-like arrangement of cells. Stain, hæmatoxylin and eosin. Magnified 30 diameters; 2 inch objective; Zeiss projection ocular No. 4.

men until it is well covered and stuck to the block. Allow the celloidin to "set" in the air a few minutes (not dry) and place the prepared block in 85 per cent. alcohol (ordinary alcohol 85 c. c., distilled water 10 c. c.), in which it may be preserved indefinitely. After six hours the celloidin will be hardened and sections may be cut, but the specimen must be kept wet during the cutting and at all times with the 85 per cent. alcohol.

The solutions required, Carnoy's fluid, thin and thick celloidin, may be made up in quantity and if *well stoppered* will keep indefinitely.

The process is simple and easily followed without special apparatus. If the specimen is small it may be collected in an 8 drachm homeopathic vial and carried in the vest pocket during the time necessary for the fixing, hardening and

embedding, which are facilitated by the body heat. Sectioning the specimen, staining and mounting are the next steps.

Clamp the block bearing the embedded specimen in the object holder of a microtome. Set the knife obliquely so that two-thirds of its edge may be used

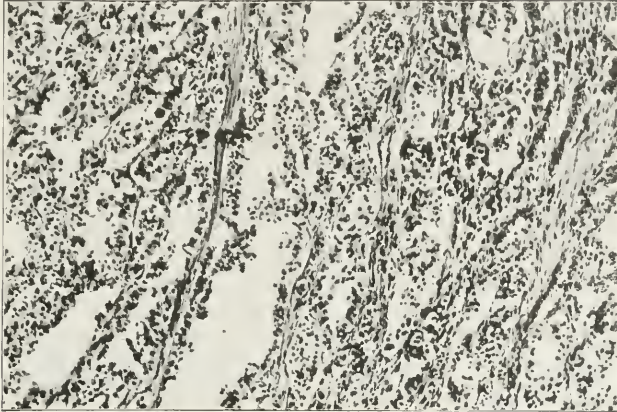


FIG. XXVI.—Carcenoma of the breast, showing invasion of cells. Stain, hæmatoxylin and eosin. Magnified 40 diameters;  $\frac{2}{3}$ -inch objective; Zeiss projection ocular No. 4.

in cutting a section. Keep the specimen wet with the 85 per cent. alcohol and the knife flooded with the same fluid. Cut the sections from ten to twenty microns thick and transfer them to a watch-glass of 85 per cent. alcohol, using a large camel's hair brush for the purpose.

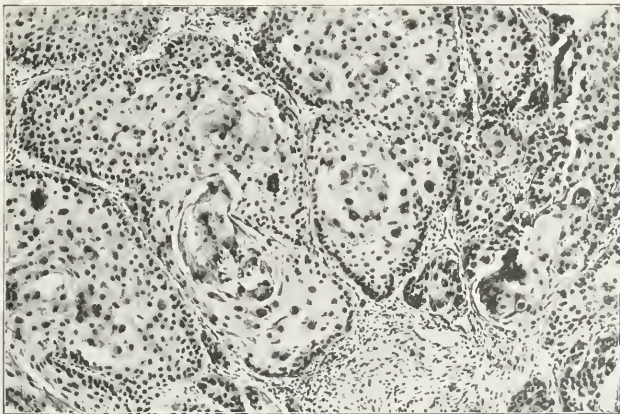


FIG. XXVII.—Epithelioma of the lip. Stain, hæmatoxylin and eosin. Magnified 60 diameters;  $\frac{2}{3}$ -inch objective; Bausch & Lomb compensating photo ocular No. 2.

If no sliding or hand microtome is at hand, satisfactory free hand sections may, with a little practice, be made. Hold the specimen in the left hand and a razor in the right. Begin at the heel of the razor and draw it towards you, rest-



ing the back on the thumb and fingers of the left hand, the edge entering the specimen. Do not try to cut a full sized section. Cut a number of sections, endeavoring to get them very thin. The specimen and razor must be kept wet with 85 per cent. alcohol. Transfer the sections to a watch-glass of 85 per cent. alcohol, selecting only the thinnest ones to stain.

*Staining.* After the sections are cut they should be stained to better show the elements. Double staining with hæmatoxylin and eosin is simple and gives good results. Transfer the specimen from 85 per cent. alcohol to distilled water to remove the alcohol. Pour this off and stain them for five to ten minutes in Delafield's hæmatoxylin which has been diluted with two volumes of distilled water. Be careful not to overstain with the hæmatoxylin. Remove and wash thoroughly in well or tap water and immerse in eosin solution made by adding eosin to water until it is of a decided pink color. Stain till the specimen is pinkish in color, wash with water and transfer to 95 per cent. alcohol (not absolute) to which a trace of eosin should be added. After the alcohol has removed the water transfer the sections to a clearing oil, as beechwood, creosote, oil origanum, oil of cedar wood, or better to the following mixture: oil of cedar wood, carbolic acid crystals and oil of bergamot, equal parts. When the section is clear and transparent transfer it to a clean slip, blot up the excess of clearing oil with filter paper, which may be pressed on the section and removed by rolling it back from one end. Apply a drop of balsam and a clean cover glass. The result is a permanent preparation ready for examination under the microscope. The washing, staining and clearing of the sections may be carried on in Syracuse watch-glasses. A section lifter should be used for transferring from one solution to another and from the clearing oil to the slip.

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A METHOD OF COLLECTING AMOEBAE.—The author of this note, being in the habit of collecting the surface ooze from the bottom of a spring hole, and putting it into pint fruit jars, noticed that as soon as the ooze settled, amœbæ were found on or near its surface. It was thought that if taller vessels of the same capacity were used so as to make the surface of the ooze in them comparatively smaller, the animals would be found in proportionately larger numbers. Therefore the ooze was collected in pint jars, and after it had thoroughly settled the surface of the ooze in the jars was lifted with a large pipette and put into test tubes, and again allowed to settle. In this way amœbæ were concentrated to such an extent that as many as fourteen were found in a single drop taken from the surface of the ooze in the test tubes, while usually several drops taken from the surface of the ooze in the jars had to be examined to find a single animal. If the animals are not to be used at once, it is well to put a few fibers of spirogyra or some other aquatic plant into each test tube. By so doing amœbæ were kept in good condition for over a week.—S. O. MAST, *School Science*.



## Laboratory Outlines for the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

### FOUR PHYCOMYCETES SHOWING VARIOUS HABITATS.

XXIV. *Mucor stolonifer* Ehrenb. Black Bread Mould. (*Rhizopus nigricans*.)

Class, Zygomycetes. Order, Mucorales. Family, Mucoraceæ.

This fungus can nearly always be obtained by placing a piece of old bread for several days in a moist chamber. An ordinary glass or jar with a cover will do very well for making the culture. Enough water should be added to keep the bread moist without soaking it too much. The fungus forms a white flocculent mass of cottony filaments (the mycelium made up of hyphæ) over the surface of the bread and later also spreads out over the walls of the glass. Some of the hyphæ will be seen to rise vertically into the air and end in rounded black heads. These are the sporangia containing the non-sexual spores.

1. Describe the naked eye characters noted above. Notice habitat and color. Notice also (1) the hyphæ passing down into the substance of the bread, (2) the horizontal stolon-like hyphæ, and (3) the upright sporangiophores.

2. Cut off a flake of the mycelium with a pair of scissors and mount in water, taking great care not to injure the delicate hyphæ. Study under low power and draw some of the hyphæ showing mode of branching.

3. Under high power draw part of a hypha and describe carefully. Any transverse septa (cross walls)? If not, what kind of a fungus is it? (Compare with *Vaucheria*.) How does this plant differ from the green algæ in general? Difference in mode of obtaining food? Why is this plant called a saprophyte?

4. Study and draw a cluster of sporangiophores showing the rhizoids at the base and the sporangia at the tips. The best are those taken from the walls of the dish. Color? Draw a single unbroken sporangium showing the columella on the inside, and the non-sexual spores. Do not mistake the columella of a broken sporangium for the entire body. Describe the structure of the sporangium. What does the columella represent? The sporangia burst readily because of the presence of an intermediate substance which swells readily in water. Of what use is this?

5. Draw and describe the non-sexual spores. Color? About how many in a sporangium?

6. This plant has a partial development of sexuality, and under proper conditions produces zygospores. If any of these are at hand or material from another species, study and draw showing the following stages:

*a.* Two neighboring, swollen branches of the mycelium, which are about to conjugate.

*b.* The stage in which the two branches have fused.

*c.* The stage in which transverse septa are formed, cutting off the apical

part of each conjugating branch. Any difference between the conjugating branches in size or contents?

d. The absorption of the wall separating the conjugating tips and the subsequent mixing of the two cœnocyctic protoplasmic masses.

e. The mature zygospore suspended between the two branches.

#### XXV. *Empusa muscæ* Cohn. Fly-cholera Fungus.

Class, Zygomycetes. Order, Entomophthorales. Family, Entomophthoraceæ.

This fungus grows on the common house fly (*Musca domestica*). In the autumn dead or dying flies attacked by this fungus may be seen with greatly swollen abdomens of a white color. Specimens may be preserved in alcohol.

1. Study a fly recently killed by this fungus, under low power without a cover-glass. Note the bands of short white hyphæ (conidiophores) protruding from between the black segments of the abdomen. Draw and describe.

2. Tear open the abdomen with needles and mount the white contents and some of the conidiophores in water. Examine under high power. Notice that the mycelium has nearly absorbed the contents of the fly's abdomen.

3. Draw some of the conidiophores with conidia still attached; also draw several of the conidia. Describe.

#### XXVI. *Saprolegnia* Sp.

Class, Oomycetes. Order, Saprolegniales. Family, Saprolegniaceæ.

This fungus can usually be obtained by placing dead flies in a dish of spring or pond water. After about five or six days the hyphæ of the fungus may be seen protruding from the body of the fly. On the tips of these hyphæ sporangia are developed which discharge numerous zoospores.

1. Notice the fly in the water, surrounded by a halo produced by the mycelium of the fungus.

2. Mount some of the mycelium in water and examine under low power. Draw a branch under high power, showing the granular protoplasm and a terminal sporangium developing zoospores. Draw a branch showing an empty sporangium.

3. Study and draw free swimming zoospores.

#### XXVII. *Plasmopara viticola* (B. & C.) B. & deT. Downy Mildew of Grape.

Class, Oomycetes. Order, Peronosporales. Family, Peronosporaceæ.

This mildew causes a destructive disease of the leaves and young shoots of the cultivated grape. The infected leaves may be collected in spring or summer and preserved in 70 per cent. alcohol or dried and kept in paper boxes. Conidial stage.

1. Examine a leaf carefully under the low power, without cover-glass. On which side do the conidiophores appear?

2. Carefully scrape off some of the conidiophores with a needle or scalpel, mount in water, and examine under low power. Under high power draw one of the much branched conidiophores. If dry material is used the conidia will probably all have dropped off. One is developed at the tip of each peg-like branch of the conidiophore.

3. Draw several conidia and describe shape, size, and color.

4. From alcoholic material cut cross sections of a part of a leaf containing the fungus, mount, and under low power note that the conidiophores come out in bunches through the stomata of the leaf. Draw.

5. To what physiological class does this fungus belong? Describe its mode of life so far as studied.

Ohio State University.

JOHN H. SCHAFFNER.

## LABORATORY PHOTOGRAPHY.

L. B. ELLIOTT.

Devoted to Methods and Apparatus for Converting an Object into an Illustration.

### Some Suggestions on the Use of the Lantern in the Class-Room.

The lantern has become one of the necessary aids in teaching, and all well-equipped educational buildings are arranged for its use. By means of pictures, tables, etc., projected on the screen, information can be imparted in an attractive manner, and a more lasting impression made than in almost any other way.

In spite of its evident value, however, it is not as widely used as one would expect, especially in the smaller institutions. This is perhaps to be accounted for by the following facts: first, because the original cost of the outfit is high; second, because of the trouble and expense of obtaining the necessary slides; and third, because the teacher usually thinks an assistant is necessary to run the lantern during the class period. On account of these and other real or imaginary difficulties, many high schools, academies, and colleges do not avail themselves of the opportunities of this method of instruction.

It is the purpose of this paper to point out how a lantern may be used for purposes of class instruction without the expenditure of a great deal of time or money.

*The Lantern Itself.* A lantern is really a very simple affair. It consists of a source of light, condensing lenses, a slide holder, and a projecting lens. These should be attached to a base in such a way that their relative position with respect to each other may be easily changed. The optical parts can be purchased from almost any dealer in optical goods, and the lamp made and the various parts mounted by anyone handy with tools. Where it is not desired to make a lantern, a good one can be purchased cheaply from any one of the many dealers in this line of supplies. A lantern should have condensing lenses four and one-half inches in diameter and a projection lens whose size will depend upon the distance the lantern is to be from the screen and the size of the picture desired.

*The Kind of Light.* Where possible, electricity should be used. This is in preference to any other form of light, since it is the most powerful and is very convenient to handle. The incandescent current can be used, and this is usually in modern buildings, or can be put in at very little expense. Where electricity cannot be secured, the calcium light can be used, but this is usually too expensive for class-room work, and acetylene or vapor light must be used. The following chart indicates the candle power of the various lights ordinarily employed.

Acetylene and vapor lights, while they do not compare with electricity for brilliancy, can in reality be made to serve very acceptably in small and well-darkened rooms and will be sufficiently effective where the classes are small and a picture over six or eight feet in diameter is not demanded.

When electricity is used it is almost invariably the incandescent circuit, because it is just as efficient and is less dangerous than the arc circuit. The 52 or 110 volt, direct, incandescent current is the most desirable. On such a current a 90° lamp gives the maximum amount of light. A hand feed lamp is ordinarily to be preferred to the much more expensive self-feed lamp.

The alternating current is sometimes the only one available and may be used very satisfactorily except for the buzzing sound which it invariably makes. One, however, soon becomes accustomed to this and ceases to mind it. With this current the carbons are best placed 180° apart, i. e., in line as shown in Fig. 2 B. They should have a soft core and should have the front surface filed flat; this brings the craters to the front and increases the amount of light as well as lessens the buzz, since the carbons may be kept closer together.

With the incandescent current it is necessary to use a resistance box or rheostat. These cost, usually, ten or twelve dollars, but by the use of a high resistance wire such as the Ia Ia No. 16, handled by Herman Boker & Co., New York, one can be easily made and will cost less than a dollar. The wire should be coiled, making a helix about an inch in diameter and eighteen inches long. This helix can be arranged between two binding posts so that its length can be varied to meet variations in the current. See Fig. 2 C.

*Method of Using the Lantern.* Usually one of the objections to the use of the lantern in the class room is the fact that unless the instructor has some one to run the lantern for him he thinks he must stand behind his audience, which is undesirable, both from the teacher's and student's point of view. This objection may be overcome by having a wide angled lens and having the lantern towards the front of the room where it can be easily operated by the speaker, who can at the same time face his audience. This is very easily done where a fair sized room is available, by having the lantern on the speaker's desk and the screen at the side of the room, as shown in Fig. 3. In this way about three-fourths of the seating capacity can be utilized and still have the pictures readily seen by all. The great

advantage of this method of using the lantern is apparent, especially when only a few slides are to be shown during the lecture period. In our lecture

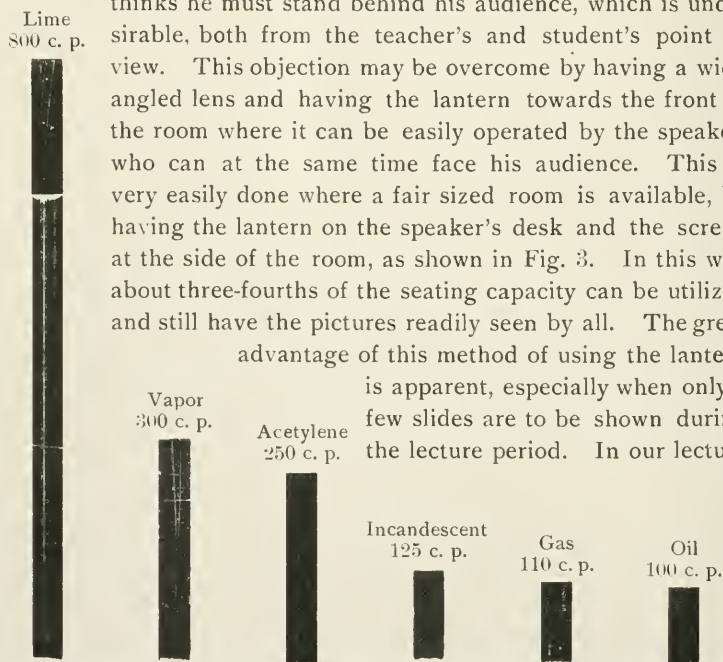


FIG. 1.—Diagram to show the relative candle power of various lights in common use.



room we have two lanterns, one at the back of the room with the screen at the front for use with large classes, and the other a small lantern, placed on the speaker's desk and throwing a picture on a side screen. We find that the work is better illustrated where it is possible to use the small lantern, because the instructor can stand before the class and show the slide at the proper time without going to the rear of the room and back again, or staying at the rear during the rest of the hour.

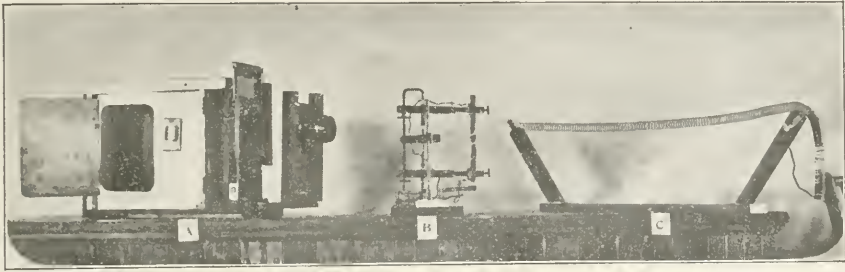


FIG. 2.—Photograph showing a home-made lantern. A shows the body of the lantern. The box is made of sheet iron, and while it is well ventilated, it is light tight, a very essential feature if it is to be used in front of the audience as suggested. a is the slide holder. B is the electric lamp taken out of the box to show its construction. C is the rheostat. The wire is held to the right hand upright in a v-shaped slot in a piece of brass, this enables one to vary the length of the coil and thus change the resistance.

Another point is in regard to darkening the room. Where rooms have been especially arranged for the use of the lantern in the day time, some method of mechanically raising and lowering the shades is provided. This is certainly most convenient, but is by no means essential. This is especially true where electricity is used. Our own lecture room is in a very old building and has only ordinary window shades. These run behind frames which have been attached to the window casings. The shades are lowered at the beginning of the hour. The room is, however, never completely darkened. Usually the curtains are left up enough to let in light for comfortable note taking. Unless the light shines directly on the screen, the picture is brilliant enough for ordinary purposes. It is a very great convenience to have incandescent lights in the room sufficient for illuminating purposes, with a switch at hand, so that at times when the pictures are not actually being shown the room may be well lighted.

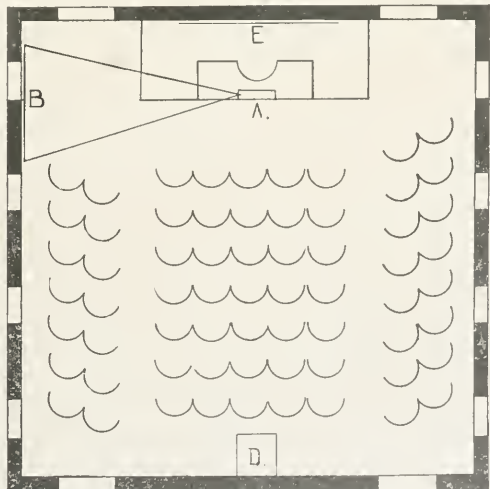


FIG. 3.—Diagram showing arrangement of the lantern in the lecture room. A is the lantern on the instructor's desk. B is the screen. D is a second lantern with a narrow angled lens to be used with large classes and throws a picture on the screen E.

*Methods of Preparing Cheap Lantern Slides.* The photographing of objects or photographic reproduction of drawing or cuts is always the most satisfactory, and any attempt to make slides in any other way is always more or less disappointing. On the other hand, the illustrations desired for a particular lecture may have no permanent value and can be used only once, or at most a few times. In such cases one does not feel warranted in going to the expense of having a photographic slide made. Again, in a lantern lecture it is not possible to use a blackboard, but it is desirable to indicate the spelling of terms, show simple diagrams, etc. Hence, methods of preparing simple, inexpensive slides may not be out of place, although they may be familiar to those who have had experience in running a lantern. Where tables of figures are to be reproduced, quotations made, etc., a stencil can be made on a typewriter and transferred to a piece of plain glass in the same way that a mimeograph copy is made. Frequently a fairly good slide may be made by moistening the glass plate with the tongue, allowing it to dry, and then writing on it with ordinary writing fluid, and when the ink is dry, rubbing lampblack over it with a piece of cotton batting. Or, again, the glass slide may be blackened by holding it over a piece of burning camphor until it is opaque, writing or drawing on it with some sharp pointed instrument, as a needle, and then dipping it in a very thin shellac, which makes the slide, if properly protected, quite permanent. The most satisfactory way, however, is to coat the slide with some transparent substance, as shellac, Canada balsam, or best of all, thin celloidin, and allow to thoroughly dry. In this case it is possible to write on the glass with a fine pointed pen and India ink. Where desirable, different colored inks may also be used. Shaded drawings may be made on a ground glass slide with a lead pencil, then the slide afterwards rendered transparent by running over it a coating of Canada balsam.

In making photographic slides a great deal of good work, especially copying, can be done with very simple apparatus. For example, a very satisfactory camera for this purpose can be easily and cheaply made, using the projection lens of the lantern for the lens of the camera, especially if this is a short focus one. The body of the camera can be made of two boxes which slide into each other, the lens being fitted into the closed end of one of the boxes and a plate holder into the closed end of the other. Some of our best slides have been made with a similar arrangement. Ordinarily the lantern slide is made for a negative and is a positive. Frequently, however, a negative is quite as satisfactory. Indeed, for tables and line drawings it is even more desirable. It looks more like blackboard work and can be seen more distinctly in a rather light room than the positive. It is also much cheaper. Where it is desired to have the lines black on a light background it can be readily and cheaply secured by making the drawings on the blackboard, photographing them, and using the negative for a slide. This method has been used very successfully by Professor Schneider of the North-western School of Pharmacy. It perhaps ought to be stated that where the negative is to be used as a slide, the pyrogallic acid developer can not be used, as it is liable to tint the slide.

W. D. FROST.

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## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Schmid, B. Beiträge zur Embryo-Entwicklung einiger Dicotyln. Bot. Zeit. 60: 207-230, pls. 8-10, 1902.

Several "pseudo monocotyledons," or dicotyls with only one cotyledon, are described by Schmid in a recent paper.

In *Ranunculus Ficaria* the single cotyledon is at first lateral but gradually becomes terminal, pushing the stem tip to one side so that it appears to be lateral. There is scarcely a rudiment of the second cotyledon. In *Bunium bulbocastanum* also there is doubt as to whether a slight protuberance should be interpreted as a second cotyledon or not. In *Cyclamen persicum* the ripe seed shows no trace of a second cotyledon.

Attempts to induce the development of the missing cotyledon by removing the prominent one, gave only negative results.

With such technique as is often described to readers of the JOURNAL it would appear that many of the points here left in doubt might have been settled.

C. J. C.

Timberlake, H. G. Development and Structure of the Swarm Spores of *Hydrodictyon*. Trans. of Wisconsin Acad. of Sciences, Arts and Letters, 13: 486-522, pls. 29-30, 1902.

It is particularly difficult to fix cells like those of *Hydrodictyon* on account of the thick wall, delicate layer of protoplasm and large central vacuole.

The following formulæ gave very good results, not only for this alga but also for *Vaucheria* and *Spirogyra*:

Merkel's Fluid,—

1.4 per cent. solution of chromic acid	-	25 c. c.
1.4 per cent. solution of platinic chloride	-	25 c. c.

Eisen's Fluid,—

0.5 per cent. solution of iridium chloride in distilled water	100 parts.
Glacial acetic acid	- - - - - 1 part.

The following stronger solution was also used.

1 per cent. iridium chloride in distilled water	-	100 parts.
Glacial acetic acid	- - - - -	3 parts.

It is unfortunate that the solutions are so expensive. Flemming's chromosmo-acetic acid mixture and also solutions containing mercuric chloride were unsatisfactory on account of the frequent distortion of the structure of protoplasm. The safranin-gentian violet-orange combination gave the most delicate differentiation. Zimmerman's fuchsin-iodine green proved good for mitotic figures, but was of little value for pyrenoids and other structures.

The nucleus has a well defined nuclear membrane and a nucleolus. In the arrangement of its chromatin it bears a further resemblance to the nucleus of the higher plants. The spindle is bi-polar and at its tips are bodies which the writer

interprets—and doubtless correctly—as centrosomes. When spores are about to be formed, a progressive cleavage takes place in the multinucleate protoplasm until the protoplasm becomes divided into very small portions, each containing a single nucleus. The cleavage is independent of nuclear division. The spores have two cilia attached to a basal body just beneath the plasma membrane. It is too early even to suggest whether this body should be homologized with the blepharoplast of higher forms. Two delicate threads connect this body with the nucleus. After the spores come to rest, the pyrenoid, which disappears at the beginning of segmentation, again becomes visible. There is no organized chromatophore.

C. J. C.

**Juel, H. O.** Zur Entwicklungsgeschichte des Samens von *Cynomorium*. Beihefte zum Botanischen Centralblatt, 13: 194-202, 1902.

Prof. Juel collected this curious parasite while travelling in Tunis. The material is quite refractory. Flem-

ming's solution was used for fixing, and the material was left in the bath in hard paraffin for a week and then would not ribbon, but had to be cut with the knife oblique, as when cutting celloidin.\*

The behavior of the megaspore-mother-cell is peculiar. At its first division two very unequal cells are formed, the one nearest the micropyle being the smaller. The smaller cell divides longitudinally and the larger one transversely, thus giving rise to a tetrad of four megaspores, of which only the one nearest the chalaza becomes functional. These peculiarities are habitual, having been observed in twenty cases. Fertilization takes place four days after pollination, and sixteen days after pollination embryos of various sizes were found. The antipodals do not divide, as has been claimed by Piroto and Longo, but may be distinguished as three undivided cells even after the endosperm has become abundant. In the ripe seed the embryo is a small, spherical mass of cells with no suspensor or differentiation into body regions.

C. J. C.

**Johnson, D. S.** On the Development of Certain Piperaceae. Botanical Gazette, 34: 321-340, pls. 9-10, 1902.

The species investigated were *Piper aduncum*, *P. medium*, *Heckeria umbellata* and *H. peltata*. The embryo-sac devel-

ops in the usual way, there being no indication of a sixteen-nucleate sac, as in *Peperomia*. In both genera there is an extensive formation of endosperm before the first division of the fertilized egg. In *Piper* the endosperm begins with free nuclear division, the walls appearing later, while in *Heckeria* walls are formed from the first. In both, the embryo is very small and undifferentiated, except that there is a rudimentary suspensor. The flowers of *Piper* and *Heckeria* are more complex than those of *Peperomia*.

In germination, the endosperm protrudes from the seed coats and continues to invest the embryo until the cotyledons and root are differentiated. C. J. C.

\* Sometimes material which infiltrates perfectly with two hours immersion in the bath, and ribbons nicely, becomes very hard and refractory after a prolonged immersion. C. J. C.



## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

**Eycleshymer, A. C.** The Formation of the Embryo of *Necturus* with Remarks on the Theory of Concrescence. *Anat. Anz.* **21**: 341-353, 1902.

The author presents some observations and conclusions obtained from the study of the eggs of *Necturus* and other

Amphibians relative to the theory of concrescence. From previous work on *Rana*, *Acris*, *Bufo* and *Amblystoma*, he concluded as follows: The cephalic end of the embryo is formed at the upper pole of the egg; the greater part of the posterior half is formed about the blastopore; the posterior end of the embryo is formed by a coalescence of the lateral portions of the blastoporic margin. Hence the greater part of the embryo arises in the darker hemisphere by differentiation in situ, and not by concrescence. A series of experiments on the eggs of *Necturus* gives the result that much more of the embryo is formed by concrescence than in the other Amphibia. The explanation of this lies in the fact that the eggs of *Necturus*, containing much more yolk material, tend to develop more meroblastically than do any other amphibian eggs. This causes a much larger proportion of the embryo to be formed by coalescence of the lateral margins of the blastopore. With the still greater amount of yolk found in most Teleosts, still less embryo is formed in situ and in Elasmobranchs and birds where the yolk mass is greatest, concrescence gives rise to by far the greatest part of the embryo, only a very limited region at the cephalic end being formed by differentiation in situ. This indicates that the primitive method of embryo formation is by differentiation in situ, concrescence being a secondary process, which has progressed *pari passu* with increase of yolk material. The methods employed were puncturing the eggs at various stages of development, and observing where the scar produced was placed in regard to the body of the forming embryo. One series in the second cleavage stage and two in the early gastrula stage were punctured, and on those that developed the above observations were made. Some difficulty was experienced, due to the easy removal of the exovates and the ease with which such large ones can be formed as to interfere with the normal development of the eggs. This makes *Necturus* a less favorable form for experimentation than most of the amphibia. E. J. C.

**Nusbaum, J., and Machowski, J.** Die Bildung der concentrischen Körperchen und die phagocytotischen Vorgänge bei der Involution der Amphibienthymus nebst einige Bemerkungen über die Kiemenreste und Epithelkörper der Amphibien. *Anat. Anz.* **21**: 110-127, figs. 1-5, 1902.

The authors have taken up the problem of the structure and formation of the corpuscles of Hassal in the Amphibian Thymus and have traced the processes these bodies undergo in their degenera-

tion, especially the part played by leucocytes in these changes. Much difference of opinion exists as to whether the corpuscles are of epithelial or of

endothelial origin or whether the lymphoid cells of the structures migrate into the bodies from without. Observations were made on adult *Salamandra maculata*, the results obtained were far superior to those from the adult frog. In the adult salamander the thymus has a large number of leucocytes, often actively dividing, as well as the usual adenoid tissue. Also bars of connective tissue, some intact blood vessels, and three kinds of structures usually called "Hassal's" corpuscles. The types of these are as follows: (1) Small, not distinctive bodies surrounding closed vessels showing both endothelial cells and a connective tissue sheath strongly developed. (2) Larger structures consisting of a single giant cell, or masses of them, or of a closed cyst of giant cells showing concentric structure. (3) Single large cells with abundant plasma crowded with larger and smaller strongly staining and refractive granules. In the development of all these structures the endothelial cells and usually the elements of the connective tissue layer of the blood vessels and the leucocytes of the thymus play an active part, while the blood corpuscles take an important but passive part, closely connected with the various changes undergone by these bodies. The small blood vessels and capillaries first change, the endothelial cells enlarge as to nuclei and plasma and push into the lumen of the vessels until they greatly reduce or entirely fill it. The vessels then break into fragments which are small and solid or larger with blood corpuscles in their cavities; later these corpuscles undergo degeneration. The small concentric corpuscles arise from the solid fragments of vessels. Those fragments containing blood corpuscles show three ways of formation of these bodies. In the first the nuclei of the endothelium and of the connective coat of the blood vessel become elongated and sickle-shaped, then fall to pieces, showing a concentric arrangement. Leucocytes may penetrate into these masses. The second product arises from those bodies containing blood corpuscles; separate corpuscles in the lumen of the vessels or in the little bladder like cavity formed in the midst of the fragment of vessel enlarge, swell, their nuclei increase greatly, even to filling the whole space of the cell, and finally the nucleus begins to take the red stain of the cytoplasm, the latter becoming at last a pale yellow. Finally the nucleus becomes pale and vacuolated and the corpuscle may disappear entirely or become a wing-shaped structure leading again to a concentric effect. These blood corpuscles are entirely absorbed by broadening leucocytes, which engulf and digest them. The last part of the paper concerns the ultimate fate of the concentric bodies, which is largely brought about by processes of phagocytosis, resulting in their complete involution. E. J. C.

**Halta, S.** Relation of Metameric Segmentation in *Petromyzon* to that in *Amphioxus* and in Higher Craniota. *Annot. Zool. Japon.* 4: 43-7, 1902.

the earlier stages the differentiation of the mesoblast is exactly parallel, while the later stages resemble the features of the higher Craniota. Thus the same animal, *Petromyzon*, shows features that make it truly a connecting link between the lower and higher types.

**Regaud, Cl., and Fouilland, R.** Paraffin Bath Heated by Electricity. *Journ. Anat. and Physiol.* 36: 574-579, 3 figs., 1900.

lighter and can be manipulated more easily. For saturating pieces, wire baskets suspended by a wire in a paraffin bath are used.

The author finds a close resemblance between the history of the mesoblast in *Petromyzon* and in *Amphioxus*, in the mesoblast is exactly parallel, while the later stages resemble the features of the higher Craniota. Thus the same animal, *Petromyzon*, shows features that make it truly a connecting link between the lower and higher types. A. M. C.

A paraffin bath has been devised to be heated by electricity. This, the inventors claim, possesses many advantages over gas and petroleum heat. It is lighter and can be manipulated more easily. For saturating pieces, wire baskets suspended by a wire in a paraffin bath are used. A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

**Bolles Lee, A.** Nouvelles recherches sur le Nebenkern et la régression du fuseau caryocinétique. *La Cellule* **20**: 181-216, 1 pl., 1902.

The material used was the testis of *Helix*, and the results are attributed in part to the use of Bouin's formol-picric

fluid, of which the following is the formula :

Saturated aqueous solution of picric acid,	-	30 pts.
Formol,	- - - - -	10 "
Glacial acetic acid,	- - - - -	2 "

Sections were fixed to the slide by the water method and then stained by Benda's iron hæmatoxylin. After decolorizing, the sections are washed in water and then stained for one and one-half minutes in 0.5 per cent. aqueous solution of acid fuchsin. This usually overstains, but is easily reduced by washing in slightly alkaline water. Ordinary tap water generally suffices. The result surpasses that obtained after the use of Flemming's classic fluid. Chromatin and the acrosome are black, while spindle fibers, the nebenkern and the cytoplasmic reticulum are red. The author finds fixation by Bouin's fluid most admirable, very uniform, and commends it highly for use in conjunction with staining method he employed.

C. A. K.

**Monti, Rina ed Monti, Achille.** Le ghiandole gastriche delle Marmotte durante il letargo invernale e l'attività estiva. *Richerche Lab. Anat. Roma e altri Lab. Biologici* **9**: 1-25 (Reprint), pls. 8, 9, 1902.

Animals were killed at intervals during winter lethargy and vernal activity. Ducts of the gastric glands and canaliculi of the delomorphous cells were

demonstrated by the Golgi method. Small pieces of the gastric wall were placed for two days in a mixture of 3 per cent. potassium bichromate (8 parts) and 1 per cent. osmic acid (2 parts), and subsequently treated with nitrate of silver. Better results were obtained with rejuvenated material. This was fixed in osmic-bichromate and afterwards kept in the simple bichromatic solution. Whenever desired the pieces are taken from the bichromate solution and placed for 5 or 6 days in a saturated aqueous solution of copper sulphate filtered and diluted one-half and renewed daily. From this they are passed to an osmic-bichromate mixture (3 per cent. bichromate of potash 9 parts, 1 per cent. osmic acid 1 part) for 24 hours and then to 0.75 per cent. silver nitrate. The impregnation of the ducts begins in 24 hours and lasts for more than 10 days. Embedding in paraffin must be done rapidly.

The Golgi-Zimmermann method was also used. Sections of Golgi material on slides are brought to alcohol and then to a mixture of 1 part 0.75 per cent. NaCl in water and 2 parts 96 per cent. alcohol for 10 minutes. They are then returned to alcohol in diffuse light for a day and subsequently stained with thionin with safranin for contrast.

Sections of material fixed in osmic-bichromate were also stained in saturated aqueous solution of rubin, washed in water, placed for a few seconds in a mixture of equal parts of alcohol and saturated aqueous solution picric acid, washed again in water and then stained for not more than half a minute in 0.5 per cent. aqueous solution of methyl-green. By this method the nuclei are stained green, the granula, especially in the delomorphous cells, red, and the protoplasm a greenish gray. An inverse coloration was produced by Galeotti's method. Sections of material fixed in Hermann's fluid were stained in saturated solution of acid fuchsin in anilin water at 60° C., then washed in water, decolorized for a few seconds in picric alcohol, washed again in water and counterstained in a 0.5 solution of methyl-green in 50 per cent. alcohol.

For differentiation of the delomorphous and adelomorphous cells, the author employed Heidenhain's sublimate (alcohol 60 c. c., chloroform 30 c. c., acetic acid 10 c. c., sublimate 7gms.) and stained sections in Mayer's hæmalum and Congo red, or Biondi's triacid, or Heidenhain's iron hæmatoxylin followed by acid fuchsin.

C. A. K.

**Hjort, J., and Dahl, K.** Fishing Experiments in Norwegian Fiords. Rep. Norweg. Fish and Marine Invest., 215 pp., 32 figs., 3 maps. Kristiania, 1900.

**Petersen, C. G. J.** Alterations and Improvements on Otter-seines for Zoölogical Purposes. Rep. Danish Biol. Sta. II: 41-45, 1902.

The otter-seine devised by Dr. Petersen (see this Journal Vol. IV, No. 11) has been redescribed by Dr. Hjort in considerable detail, and he commends its utility for zoölogical collecting. The difficulty caused by the net

cutting into soft bottom was overcome by raising the ground rope a few inches from the bottom by means of glass floats attached to cords about six inches in length. These cords were fastened to the ground rope and also to light sinkers. The sinkers follow irregularities in the bottom, and the floats keep the ground rope free from the ooze. Small seines constructed on the pattern of the otter trawl, with silk gauze or bobinet for mesh, were used in shallow water and for surface fishing. For deep water a small bag with fine mesh was placed outside and behind the bag of the main seine, and served to retain the smaller organisms without carrying the strain of the heavy load, or in any way decreasing the effectiveness of the coarser seine.

Dr. Petersen records the use of his otter-seine in many seas with uniform success, but suggests that, owing to complications in hanging the seine, the pattern seine be purchased from Danish makers, and offers his services to intending purchasers. The cost of a complete net is about \$25.00. He has found that the trouble caused by the seine cutting into soft bottom may be obviated by cutting down the length of the arms to mere stumps and widening the mouth of the seine by stretching the meshes upon the elongated head and foot ropes. The twisting of the crow-foot, caused by turns in the tow-rope, was prevented by using shackles with ball-bearings. Advice is also given for maneuvering the vessel in lowering and raising the seine, and suggestions are made for keeping the seine upon the bottom.

C. A. K.



## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoological Laboratory,  
University of Michigan, Ann Arbor, Mich.

**Lee, F. S., and Salant, W.** The Action of Alcohol on Muscle. *Amer. Jour. Physiol.* 8: 61-74, 1902.

The method of experimentation used in this work was to ligate one hind leg of a frog and then inject into the dorsal lymph sac or the stomach a quantity of a solution of ethyl alcohol in distilled water. The alcohol thus entered the circulation and reached all parts of the body except one hind leg. The non-alcoholized leg was immediately amputated and its gastrocnemius prepared in the usual way for stimulation. In all cases the muscle itself was stimulated by induction shocks until completely exhausted, the contractions being recorded on a slow drum. In twenty to seventy-five minutes (majority, forty-five minutes) the frog was killed and the alcoholized gastrocnemius prepared and stimulated just as the normal muscle had been. The rate of absorption of the alcohol was found to vary greatly in different individuals, so that the amount of solution injected into the lymph sac or the stomach does not precisely determine the relative amount of alcohol which the muscle shall receive. The strength of the solution was more important, strong solutions producing specifically different effects from weak.

In small quantities (.03 c. c. of 10 per cent. alcohol per gram of frog) alcohol had no observable effect on the muscle. In medium quantity (e. g., 40 parts by weight of pure alcohol to 1000 parts of body weight) alcohol has a favorable action. The muscle is able to contract more quickly, relax more quickly, make a larger number of contractions and perform a larger amount of work in a given time. The working time of the muscle—*i. e.*, the time before fatigue occurs—is longer. The action of the alcohol is shown by experiments on curarised frogs to be directly on the muscle protoplasm and not on the intra-muscular nerve tissue. A large quantity of alcohol has an unfavorable action on muscle, its effect being essentially the reverse of that produced by medium quantities of the drug.

R. P.

**Atwater, W. O., and Benedict, F. G.** An Experimental Inquiry Regarding the Nutritive Value of Alcohol. *Natl. Acad. Sci.* 8: Sixth Mem., 231-397, 1902.

In this paper the authors give a complete report of their work on the nutritive value of alcohol. The work has been carried on for a number of years

and preliminary reports of parts of the results have already been published. The experiments were all conducted with healthy, adult men as subjects and the general method followed in the experiments was to study quantitatively and precisely by means of the "respiration calorimeter" the metabolism of matter and energy in the human body, when a moderate amount of alcohol formed a regular portion of the diet.

The amount of alcohol given per day to the subjects during the course of

the experiments was about 1 gr. per kilogram of the body weight, and of this amount of alcohol over 98 per cent. was oxidized in the body. In these experiments the alcohol was more completely consumed than are the nutriments of ordinary mixed diet. The potential energy of the alcohol oxidized in the body was transformed completely into kinetic energy and appeared either as heat or as muscular work, or both. The alcohol was essentially as effective in protecting body fat from consumption as were isodynamic amounts of fats and carbohydrates. The efficiency of alcohol in protecting body protein was evident, although the results on this point were not entirely uniform with all the subjects.

R. P.

**Ramsden, W.** Some New Properties of Urea. Proc. of the Physiol. Soc., pp. xxiii-xxvi, 1902.

A series of experiments on the action of urea on proteid substances has brought to light a number of hitherto unknown facts, some of which have important bearings on general laboratory technique. The following are some of the more important results. The presence of urea up to saturation prevents the coagulation by heat of all proteid solutions examined. Globulin, caseinogen, acid, and alkali-albumen, copper albuminate, and even heat coagulated proteids swell up and dissolve in a saturated aqueous solution of urea. Dry gelatin is dissolved at room temperature until 40 per cent. is in solution. Coagulable proteids are converted at room temperature into a substance possessing all the properties of alkali and acid-albumen according as the action of the original proteid solution was alkaline or acid. Urea has a marked accelerating effect, greater as the amount increases up to about 10 per cent., upon the digestion of fibrin by pepsin, HCl (.3 per cent. HCl), or by trypsin. In much larger quantities it has a retarding influence. "A dead frog placed in saturated urea solution becomes translucent and falls to pieces in a few hours. The ligaments, tendons and connective tissue throughout the body are converted into a clear, soft jelly. The muscles if shaken briskly in water fall completely into individual muscle fibres, which retain their structural features and make admirable histological preparations. The cornea swells up and becomes soft, the lens is extruded from the eye on slight pressure. The hæmoglobin of the blood is converted into a body giving the spectrum of alkaline hæmatin, and which on reduction with ammonium sulphide gives the spectrum of hæmochromogen. The skin brushes away with the slightest touch. Nervous tissues become semi-transparent and the nerves readily rupture. Connective tissues of different animals are differently affected. In a saturated urea solution no putrefaction ever takes place." This action of urea on connective tissue makes it a valuable histological reagent for the separation of a tissue into its individual elements (e. g., cardiac and skeletal muscle fibres, fat cells, etc.). The tissues can be preserved indefinitely in the saturated urea solution, and only requires transference to water for a short time to become stainable by the ordinary methods.

Various compounds of urea with fatty acids were studied. Further investigations regarding the reasons for and method of the influence of urea on proteids are being carried on by the author.

R. P.

**Bilharz, A.** Die Lehre vom Leben. Weisbaden (J. F. Bergmann), pp. xiv and 502, M. 10, 1902.

An extended, purely metaphysical discussion of biological problems.

R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt,  
Harvard University Medical School, Boston, Mass.

**Brinckerhoff and Tyzzer.** On the Leucocytes of the Circulating Blood of the Rabbit. *Journal of Medical Research*, 7: 173-189, 1902.

There are five varieties of leucocytes in the peripheral blood of the rabbit.

I. Lymphocytes. These form 45 to 55 per cent. of the total number

of leucocytes. Size, 7 to 9  $\mu$ . Nucleus, round; chromatin granules in masses, having a mural arrangement. Protoplasm, non-granular and strongly basophilic. They originate in the germ centers of the lymph-nodes.

II. Large mononuclears. Two to eight per cent. of total number. Size, 12 to 16  $\mu$ . Nucleus, oval or curved, vesicular. Protoplasm, non-granular, slightly basophilic. The writers think these cells arise from the endothelium of the lymph and blood vessels.

III. Amphophiles. These are the homologues of the neutrophilic leucocytes of human blood. Ehrlich defines them as cells possessing granules which take either the acid or basic dye. The writers, however, were not able to stain the granules with the basic stain. Forty to fifty per cent. of the leucocytes belong to this class. Size, 10 to 12  $\mu$ . Nucleus, polymorphous; chromatin, in masses, murally arranged. Protoplasm, granular; granules small, ovoid, oxyphilic. They arise from the amphophilic cells of the bone-marrow.

IV. Eosinophiles. One-half to one per cent. Size, 12 to 14  $\mu$ . Nucleus, polymorphous; chromatin, in masses, murally arranged. Protoplasm contains large ovoid, oxyphilic granules. They are formed from the eosinophilic cells of the bone-marrow.

V. Mast-cells. Four to eight per cent. Size, 10 to 12  $\mu$ . Nucleus, polymorphous, poor in chromatin. Protoplasm contains small, spherical, basophilic metachromatic granules.

In making dried blood films, a small drop of fresh blood was touched with the edge of a clean cover-slip. The edge of this cover, with the drop of blood adhering to it, was pressed against the surface of a second cover-slip, the two making an angle of about forty-five degrees. Capillary action spread the drop of blood along the angle formed by the two glass surfaces, and the blood was then evenly distributed on the second cover by sweeping the first across its surface. This procedure yielded more satisfactory results than the common method of Ehrlich.

J. H. P.

**Nicholls.** Simple Adenoma of the Pancreas Arising from an Island of Langerhans. *The J. of Med. Research*, 8: 385-395, 1902.

Primary tumors of the pancreas have only occasionally been noted. Segie, in eleven thousand five hundred au-

topsies at Milan, found only one hundred and thirty-two instances, a proportion of one hundred and fourteen-hundredths per cent. In these cases carcinoma was observed one hundred and twenty seven times; sarcoma, twice; cysts,

twice: syphiloma, once. Förster, in six hundred and thirty-nine post-mortems, found eleven cases of carcinoma, a proportion of nine-tenths of one per cent. Benign growths of the pancreas have received very scanty mention. Nicholls describes such a tumor which he accidentally discovered at autopsy. It was a small, round and somewhat flattened nodule on the anterior surface of the pancreas, situated about the junction of the middle and terminal thirds. It was tawny yellow in color with a few distended blood vessels upon its surface. On section it seemed to be well circumscribed, rather soft, and exuded a little blood. Microscopically it appeared as a perfectly oval nodule in the pancreatic substance, measuring three by two and a half millimeters. It was completely enclosed in a connective tissue capsule which contained blood and lymph sinuses. In the capsule small groups of cells were also seen resembling those of the pancreatic acini, but flattened, compressed and atrophic. The tumor was composed of a great number of cell-masses bounded by more or less completely anastomosing bands of fibrous tissue so that a somewhat imperfect and irregular alveolar looking stroma was produced. The cells in these alveolar spaces resembled the pancreatic cells and the growth looked like the tubular adenomata of the kidney. The stroma was composed of slightly cellular connective tissue with round or bluntly spindle nuclei. Minute blood vessels were found in the bands of the stroma. The blood sinuses proved to be not true sinuses. The cells varied somewhat in shape according to their arrangement in columns or in masses, being short columnar or polyhedral. The cytoplasm was granular, the nuclei were pale and somewhat vesicular, generally round or irregularly oval, and rather large. The capsule was nowhere invaded. It was consequently diagnosed as a simple adenoma. No growth like this has been found reported.

Nicholls reviews the literature and classes some of the cases reported as adenomata, as carcinomata. Thierfelder's, Neve's and Ruggi's cases can thus be excluded. A Cesaris-Demel has reported a case, however, of true adenoma of the pancreas which had its origin from the excretory ducts. Biondi also has published a case which arose from the pancreatic ducts. Some hemorrhagic cysts have been described that developed within an adenomatous overgrowth. In Nicholls' case the cells did not resemble the ductal epithelium since they were granular with a more deeply staining cytoplasm and showed no true lumina formation. He thinks the arrangement of the glandular cells, their structure, their peculiarities of staining as well as the appearance of the fibrous stroma, all tend to confirm the idea that the tumor originated in the overgrowth of an Island of Langerhans.

W. R. S.

Michaelis, L. Ueber Mastzellen. Münchener med. Woch., pp. 225-226, 1902.

It is not generally known that the granules of mast-cells are soluble in water. The mast-cells, which are present in such very small numbers in normal blood, have more resistant granules than the mast-cells of leukæmic blood. In fifty per cent. alcohol the writer found that the granules remained intact. He has devised the following staining method:

1. Fix the films by heat or alcohol.
2. Stain five minutes, or more, in a saturated solution of thionin in fifty per cent. alcohol.
3. Wash off the stain quickly with fifty per cent. alcohol.
4. Dry.
5. Mount in Canada balsam.

The mast-cell granules stain reddish brown to reddish violet; the nuclei blue.

J. H. P.



## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn, Wesleyan University, Middletown, Conn.

Harris. Concerning an Improved Method of Making Collodium Sacs. *Cent. f. Bac. u.* Par. I, O, 32: 74, 1902.

The advantage of the use of collodium sacs for bacteriological experiments has come to be very thoroughly recog-

nized, but their use has been more or less prevented by the difficulty of making them successfully. Harris describes a new method of preparing them which simplifies all previous methods, and makes the preparation of a collodium sac an extremely simple thing. For accurate description of the method one must refer to the original article, but it is briefly as follows:

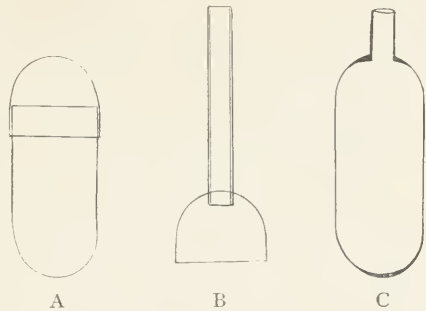
Large gelatin capsules are obtained from a druggist (Fig. A), and while closed, a glass tube is heated and thrust through one end of the capsule for a short distance (Fig. B). This is soon fixed in position by cooling of the gelatine and serves as a convenient handle for manipulation of the capsule. The capsule is then coated by being placed in a thick syrupy solution of collodium, the collodium being allowed to coat both the capsule and about one cm. of the glass tube. It is then removed and allowed to dry. There is thus produced a collodium sac on the outside of the gelatine capsule. The removal of the gelatine from the interior is a simple matter. The capsule is filled with and is immersed in hot water. The heat soon dissolves the gelatine, and then the solution may be poured or drawn out of the capsule by a pipette, leaving the collodium sac fastened to the end of the glass tube. The sac may then be filled with any nutrient solution and the glass tube sealed in a flame if desired (Fig. C). It is possible to remove the gelatine capsule in the process of sterilizing, if desired, by simply filling the capsule with a nutrient bouillon after coating it with collodium, immersing it in a dish containing bouillon and then sterilizing it in an autoclav at 120°C. With such treatment the gelatine is both melted and expelled from the collodium sac, leaving the sac filled with the sterilized nutrient fluid. Such sacs may be conveniently used for demonstrating dialysis in class experiments.

H. W. C.

Linossier, G., and Lemoine, G. H. A Medico-legal Investigation of the Source of Blood by the Use of Precipitant Serums. *Bul. de l' Acad. Med.*, March 25, 1902.

A blood-stain being given, the determination of its source is one of the problems most frequently met with in legal-medicine, and one of the most

difficult of solution. Until recently the only available method consisted in dilut-



ing the stain with a suitable liquid and measuring the diameter of the corpuscles remaining intact. In practice this method furnished no means of distinguishing the nucleated and elliptical corpuscles of birds from the discoidal ones of the mammals.

It may sometimes, however, furnish a means of distinguishing human blood corpuscles from very small ones, such as occur in the kid; but human blood can never be distinguished by this method from that of the dog or rabbit.

The method proposed by Uhlenluth, which is based upon Bordet and Tschistowitch's use of precipitant serums, is one of extreme delicacy. The authors have studied the precipitant serums from an entirely different point of view, and have been led to make a number of interesting investigations from the medico-legal standpoint. The most important of these experiments relates to the specific characters of the precipitant serums. All authorities agree that, with the exception of certain cases in which confusion exists between the blood of closely related animals, these serums are strictly specific. For example: the precipitant serum for human blood precipitates only human blood and the blood of apes, and no other that could possibly be confused with them. The use of precipitant serums is founded upon this specific characteristic. But from the investigations of Linossier and Lemoine it appears that this characteristic does not exist. These authors have however, established the fact that the precipitant serum for human blood precipitates also the blood of cattle, horses, dogs, sheep, pigs, guinea-pigs, chickens, etc., and the reaction is incomparably more delicate with human blood. It is possible to avoid, in advance, the possibility of error which arises from this source, by conducting the experiment only in diluted blood solutions. A solution of serum (one in one thousand) has always been found to be precipitated by the corresponding active serum, and never by any other. This possibility of error being guarded against, the new method of investigation into the source of blood by the use of precipitant serum will furnish invaluable aid to legal medicine.

A. GIRAULD.

Trans. by Eleanor L. Lattimore.

**Rohnstein.** Eine einfache Konservierungsmethode für die Zwecke der klinisch-mikroskopischen Diagnostik. Fort. d. Med., No. 2, 1902.

There has long been felt the need of a satisfactory means of preserving organic material containing cellular elements and bacteria, like sputum, etc., for subsequent microscopic study, which will preserve the morphological elements indefinitely. Various plans have proved more or less unsatisfactory, and Rhonstein has developed a method by which the material is first sedimented, after which there is added to it a material which has the power of fixing and preserving the albuminoid holding material. This avoids the difficulty of all manipulation and preserves the morphological elements uninjured. The material used by the author for such preservation is a glycerine solution of formaldehyde, and he has used it successfully in the preservation of sputum, urine, feces, and the contents of the stomach.

H. W. C.

## QUESTION BOX.

Inquiries will be printed in this department from any inquirer.  
The replies will appear as received.

No. 28. Why do some crystals polarize and some not; e. g., common salt, etc?—J. L. L.

No. 29. What is a good method for making sections of moss leaves, etc?—  
W. B. D.

### REPLY TO QUESTION No. 24.

The following method for Fixing and Flattening Paraffin Sections is a modification of Heidenhain's, and may be found useful where sections are apparently hopelessly crumpled. Dr. H. E. Durham (Quart. Jour. Micro. Sci. **33**: 1892) says he finds it better and easier than Gaskell's method:

1. Place sections on a dry slide or one moistened with methylated spirit diluted to 70 per cent. alcohol.

2. Place slide on warming plate just hot enough to soften, no more—useless ribbon sections make a good "thermoscope."

3. With a pipette add a *few drops* of 70 per cent. alcohol; as the paraffin softens the wrinkles disappear. When the sections are flat, remove excess of alcohol with the pipette (avoid too much alcohol, or sections are displaced; avoid too little alcohol, or they will not become flat).

4. Evaporate the alcohol; warm the paraffin; dissolve paraffin with xylol, benzol, or 7-8 parts benzol to 1 part of turpentine; mount in Canada balsam.

If staining on the slide is desired, Dr. Durham recommends alcohol for fixing rather than any other, because no stained film is left as with collodion, albumen, etc. For other notes see Gage's book on The Microscope, Transactions of the American Microscopical Society, Lee's Vade Mecum, etc.—V. A. L.

### REPLY TO QUESTION No. 27.

The formula for Knop's solution, as given by Dasseville,\* is as follows:

Calcium nitrate, - - - - -	1.00 g.
Mono-potassium phosphate, - - - -	0.25
Potassium nitrate, - - - - -	0.25
Magnesium sulphate, - - - - -	0.25
Ferric phosphate, - - - - -	trace.
Water, - - - - -	1000

Instead of ferric phosphate, I have used in this culture solution a trace of ferric chloride, which appears to work a little more satisfactorily. A method for preparing a concentrated solution of the same nutrient salts is found in a paper by Knop in Landw. Versuchs-Stationen, Bd. XXX, p. 292-294, 1884.

University of Tennessee, Knoxville.

SAMUEL M. BAIN.

\* Rév. gén. de Botanique, t. VIII, p. 285, 1896.

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LABORATORY workers are becoming more and more alive to the possibilities of photography as a practical assistant in demonstration, record, and investigation work.

The chief objection to photography for laboratory purposes has been based upon the inaccuracy and undeveloped condition of apparatus which has been at the disposal of laboratory workers, and of the lack of processes easily workable in the laboratory for getting the desired results.

The recent efforts of the manufacturers of optical apparatus in all lines, in the production of improved types of photomicrographic cameras, improved lenses for photomicrographic work, and more suitable projection instruments for using the results of photography in the lecture room, in the form of lantern slides, has given a great impetus to laboratory photography in all its branches. More attention is now being given to the photographing of what may be termed macroscopic objects, that is, objects which while too large for observation under the microscope in the ordinary way, are still small enough to require at least a pocket magnifier for their examination, and it is for photography in this class of objects that there is a wide field for improvement in apparatus generally, and in the methods of photographing. Our knowledge of many familiar objects as, for example, the house-fly, would be immensely enhanced by the ability to make photographs of ten, fifteen, or twenty diameters enlargement with facility, photographs which would represent the creatures as they really are instead of the flattened out macerated silhouettes of their external coverings usually found in the collections of microscopists under the title of prepared insects.

Photographs by transmitted light are necessary for many things, but there is an immense and unexplored field in the photographing, with medium and low powers, of transparent as well as opaque objects by reflected light. With the proper methods of illumination and lenses of proper foci and aperture, it is possible to obtain just as good and natural looking photographs of these small things as it is of large objects.

The biological laboratory has only in recent years had such a luxury as a photographic dark-room. What is needed quite as much for modern photographic work in the laboratory is a photographing room where the light conditions can be controlled, and where the electric arc light can be used for artificial illumination of objects when desired, so that when objects of any kind are to be photographed by reflected light, they can be lit in the proper manner to bring out the form of the object and its detail so as to give it a natural appearance. How many times we see photographs of such objects as fossils, shells, plants, etc., which, while having all the detail which one could desire, are yet as flat and unlike the objects themselves as anything could well be.

While it may be possible to make properly lighted pictures, having full detail, of natural objects without any special adaptations, the time saved, and the superior results obtained, if a studio room could be had in the laboratories, would amply repay the extra expense. In the new laboratories which are being planned, the biologist should, by all means, insist upon a properly located photographing room in which both macroscopic and microscopic photography can be done, and adjacent to it a well and properly equipped dark-room. Such an addition will do more toward profitable demonstration in the institution than the employment of an extra instructor or demonstrator could do, and at a far less expense.



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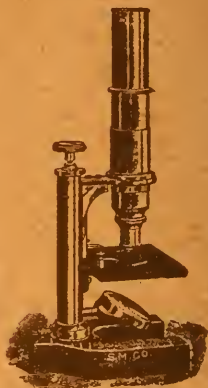
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# Journal of Applied Microscopy and Laboratory Methods

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## Bacteriology for High Schools.

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### I.

It would seem very desirable to give pupils in the high school some definite ideas in regard to the bacteria. Everyone ought to know what bacteria are, what they do, where and how they live, and the relation which they bear to man's welfare. No one can, however, get any adequate idea of them without seeing them, since there is no familiar object with which they can be compared. The rapidity of their multiplication and the changes which they are capable of bringing about are best appreciated when they can be seen and studied growing on artificial media. It would seem desirable, then, to have the pupils actually do some laboratory work in bacteriology. But it is not worth while to have them spend the time necessary to work out the methods ordinarily used, since the aim should be to give the pupil the proper idea of bacteria and some notion of their activities and not to impart technical methods. This means that they are to be taught the means of demonstrating the bacteria in various places, their action on certain substances, and the effect of various agents in modifying or destroying their activities. With this idea in view the following series of exercises have been written out. It is believed that they will be in line with a need that has already been felt in many quarters and it is hoped that they will create an interest among a larger circle of teachers. For it is certainly true that the masses can not long be content to remain ignorant of the general principles of sanitary science, a science which has done so much in the last quarter of a century to increase the sum total of human happiness.

### LABORATORY ROOM.

For this course it will not be necessary to have a special room or any expensive equipment, except a microscope which must be furnished by the school or loaned by an interested physician. The work can be done (and preferably in most cases) at home with little more than the ordinary kitchen equipment. There are, however, a number of schools where a course like this can be given a regular place in the curriculum either as a distinct course or a part of a

course in physiology, botany or general biology. For these schools, and it is to be hoped that their number will be constantly increasing, it will be desirable to equip a room where this work can be advantageously done. The size of the room will depend upon the number of students to be accommodated at any one time. Professor Minot, in discussing the unit laboratory plan, has recommended  $17\frac{1}{2}$  square feet per student. The room should be well lighted and should face north or east if possible. The tables can be of any convenient size or form. Where a number of students are to be accommodated at a single window, the truncated form is very satisfactory. Plate glass resting on a rubber mat is perhaps the ideal top, but can rarely be afforded. The next best is a wood top, either a soft or hard wood, stained with anilin black. (See JOURNAL OF APPLIED MICROSCOPY, Vol. I, p. 145.) This top will withstand the action of chemicals, even strong sulphuric acid for a short time, wears many years, and can be applied to an old as well as a new top after the old finish has been planed off. The room should be supplied with water and have sewerage connections and, if possible, be supplied with gas.

#### APPARATUS.

*Sterilizers*—Two kinds of sterilizers will be necessary, one in which hot air is used and which can be heated very hot. This is called a hot air sterilizer or oven. Such a sterilizer

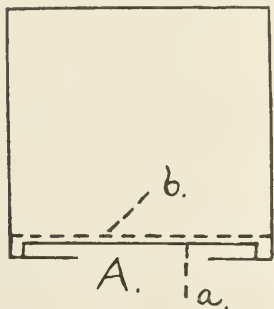


FIG. 1.—Simple hot air sterilizer made from a tin cracker box. A, Circular opening in side of box. a Copper plate on short legs. b Perforated iron grate.

may be purchased, adapted to the needs of any laboratory, or one can be improvised. The oven in a kitchen stove may be made to serve. A gas or gasolene stove may be used in the laboratory. Or simply the oven of such a stove may be rigged on an iron support and heated by a Bunsen burner or, where gas is not available, a Primus or similar kerosene or gasolene lamp may be used. A more simple form may be made from a tin cracker box which has had a circular hole cut in one side and then a copper plate fitted over this inside on short legs, which is of the same shape but slightly smaller than the side of the box. The flame plays directly against this copper bottom. A

grate should be placed about an inch above this plate. It may be made of perforated iron (Fig. 1). The whole box should be covered with asbestos and may be heated with a Bunsen burner or a Primus lamp. In this form of sterilizer will be heated glass and metal apparatus, but for culture media especially a steam sterilizer will be necessary. This can be purchased, and the form most widely used is the Arnold steam sterilizer. A very simple yet efficient form, however, may be made with a covered pail. The pail is perhaps best made of galvanized iron, but a tin one

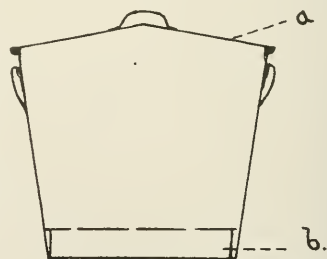


FIG. 2.—Simple steam sterilizer consisting of a galvanized iron pail with a cover a and a false bottom b.



of any size serves just as well until it rusts out. It is only necessary to provide a perforated grate. This grate should be far enough from the bottom so that the space between them will contain enough water to last an hour. (See Fig. 2.)

*Media cooker.*—A double boiler or rice boiler. (Fig. 3.)

*Graniteware* bread pans with a piece of window glass for cover.

*Funnel.*—A three or four inch glass (or graniteware will do) funnel.

*Tin cup.*

*Small tin pail.*

*Flasks.*—Erlenmeyer form. (Fig. 4.) Two sizes, liter and two hundred and fifty cubic centimeters capacity. Bottles may be used, especially if thin glass ones are obtained, such as whisky flasks.



FIG. 3.—Media cooker.



FIG. 4.—Erlenmeyer flask.

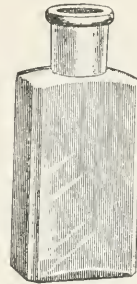


FIG. 5.—Wide mouth  
Blake bottle.

*Test-tubes.*—If these are purchased thick walled ones are best and should be made of a kind of glass which will stand continued and high heat. Size,  $\frac{5}{8} \times 5$  inches. Homeopathic vials may be used, the straight form, *i. e.*, without necks, are best. The test-tubes are best sterilized in wire baskets which are made for the purpose, but the tin pails enumerated above may be used.

*Petri dishes.* These should be about four inches in diameter (Fig. 6). Bottles which have large flat surfaces may be used; such as wide mouth Blake, 8 oz. capacity (Fig. 5).

*Glass tumblers.*

*Medicine droppers*, or glass tubing from which they may be made.

*Thermometer*, 0–250°C.

*Potato knife*, or case knife.

*Measuring glass*, or graduated cylinder.

*Test-tube rack.* A wooden block with holes bored in it will do.



FIG. 6.—Petri dish.

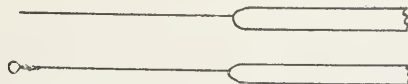


FIG. 7.—Platinum needles, showing part of  
the handles.

*Microscope and accessories.* A microscope magnifying about 500 diameters can be used very satisfactorily in this work, but of course only the larger bacteria can be sharply outlined. An oil-immersion objective is a desideratum. A

$\frac{1}{10}$  inch is about 20 per cent. cheaper than the usual  $\frac{1}{12}$  inch and is satisfactory for all ordinary bacteriological work. A substage condenser and iris diaphragm are necessary if an oil-immersion objective is used.

*Slides and cover-glasses.* Only a very thin cover-glass can be used. A glass 0.17 mm. thick or a No. 2 Bausch & Lomb are the thickest that can be used, since with the thicker ones it is impossible to use the oil-immersion objective.

*Platinum needles.* Two inches of No. 27 platinum wire should be fused into glass rods for handles (Fig. 7). These needles are for handling the bacteria and will be much used. It is probable, however, that in this course all the work required can be done with ordinary "hat pins."

*Gummed paper.* For labels or a wax pencil made for writing on glass may be used.

#### CHEMICALS.

*Extract of beef.* Leibig's or similar grade.

*Peptone.* The dry form.

*Sodium chloride.* Common table salt.

*Soda.* Baking soda or saleratus.

*Gelatin.* The gold label sheet gelatin is best, but the commercial article, such as Knox, can be used.

*Dextrose,* or grape sugar.

*Absorbent cotton.*

*Cotton wool.* A good grade of cotton free as possible from foreign matter.

*Litmus paper.* Blue and red.

*Filter paper.* Either in sheets or circular, of a size to fit the funnel.

*Anilin dyes.* A saturated alcoholic solution of fuchsin or methylen blue.

*Corrosive sublimate tablets.*

*Canada balsam.* Dissolved in xylene (xylol) and about the consistency of oil.

*Immersion oil.* This will probably be supplied with the oil-immersion lens and will not be needed except for use with such an objective.

A more complete list of bacteriological apparatus will be furnished by any of the dealers. In making out any list it is desirable to have a catalogue issued by such firms as: Bausch & Lomb Optical Co., Rochester, N. Y.; Eimer & Amend, New York City; Leitz, Chicago, Ill.; Richards & Co., Chicago, Ill.

Bacteriological Laboratories, University of Wisconsin.

W. D. FROST,  
E. G. HASTINGS.

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IMPROVED HOT AIR AND STEAM STERILIZERS.—If one has access to a kitchen oven after cooking or baking, the temperature is quite high enough for sterilizing dishes, test tubes, pipettes, flasks, cotton wool, etc. Cotton wool should be put inside a beaker, and when it is slightly scorched the articles may be considered sterile, or, if a thermometer is used, when they have been exposed for one hour to a temperature of 150° C. For a steam sterilizer for culture media nothing more is necessary than an ordinary medium-sized kitchen pan half full of water boiled over a ring gas-burner, and provided with a potato-steamer, the sides of which may be covered with ordinary felt in order to conserve heat as much as possible, and the lid, if close fitting, should have three or four small holes made in it, so that there may be direct circulation of steam.—*Jour. State Med.*, 11: 2.

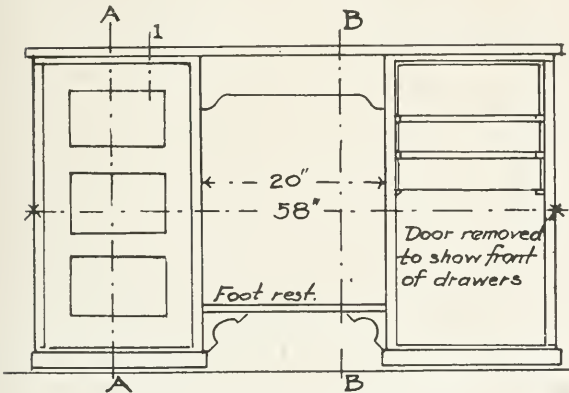
## A Combined Locker and Laboratory Table.

*Specifications.* Both sides of the table are to be exactly alike. Each table will then have four doors, four drawers each five inches deep in the clear, and eight drawers each three inches deep in the clear.

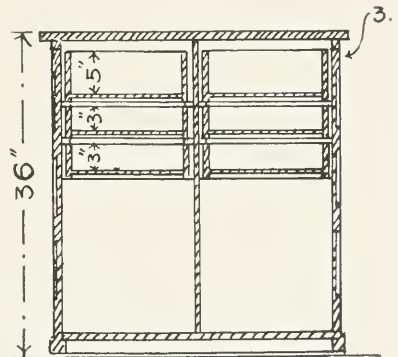
Exterior of tables and fronts of drawers are to be of selected red oak ; drawer guides or slides of oak, maple, or cherry, and balance of interior work of poplar.

Each door shall be hung with one pair good brass fast pin butts, and shall be fitted with an "Anti-dial" combination lock. Each table shall be fitted with eight "standard" No. 7, all steel castors.

Except the top all exposed work, including drawer fronts, shall be filled with silica paste filler, and shall then be finished with one coat of white shellac and one coat of Johnson's, or equally good wax. Inside and drawers, except fronts, shall have one coat of orange shellac.



ELEVATION OF SIDES.



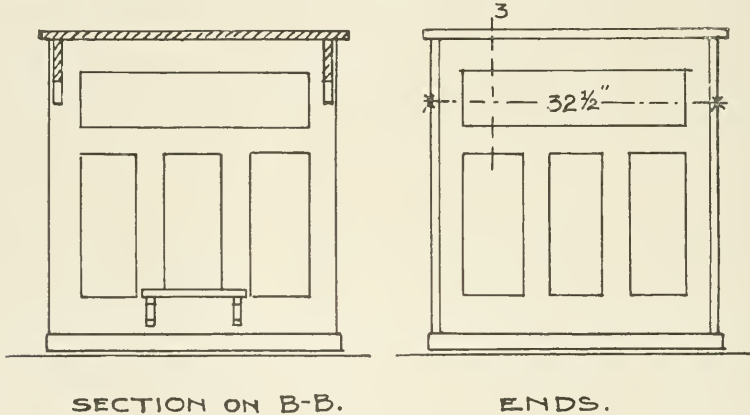
SECTION ON A-A.

The table in question was designed for laboratory work in Physiology and Materia Medica. The height and also the area of the table top is somewhat greater than ordinary for the reason that in experimental physiology it is necessary at times to have considerable apparatus upon the table and the height is desirable because in some experiments the student can do his work better standing than sitting. The foot rest attached to the tables, in connection with a stool a trifle higher than usual (24 inches), enables the table to be perfectly serviceable and entirely satisfactory for all forms of work at which it is desirable that the student should sit.

The chief advantage of the table, however, is believed to rest upon the fact that a considerable economy of space and convenience to the worker is subserved. The floor space covered by the table, in many instances, is not utilized at all, except for the work done upon the top of the table. Lockers, when necessary, have been built along the walls of the laboratory or in the hallway, or in an adjoining room, thus taking up space which might be profitably utilized by wall cases containing specimens, models, or general apparatus bearing upon the

laboratory course. Students often pass to and fro from table to locker, causing more or less jar and vibration, especially annoying if microscopical work is going on. Such an arrangement is doubly inconvenient. It is annoying to the student to be obliged to go from table to locker. It is also annoying to his fellow workers to have him do so.

The combined lockers and table obviates these disadvantages. Each table contains four lockers and two students can work at one table and have their apparatus right at hand. Twelve tables will provide lockers for forty-eight students and twenty-four students can work at the tables at one time.



The table would appear to be useful for biological work in general, although in certain cases a proportionate change in dimensions may be desirable.

The cost of the combined locker-table is less than the total cost of a table and four lockers built separately. In lots of one dozen, the combined locker-table including a combination lock for each locker can be built in red oak for fourteen dollars each, or in chestnut for twelve dollars each. The writer has used these tables for nearly two years and has found them satisfactory in every way.

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USEFUL BOOKS IN ELEMENTARY PLANT PHYSIOLOGY.—Barnes' *Plant Life*, containing particularly practical directions for simple experimenting. MacDougal's *Elementary Plant Physiology* and Ganong's *Laboratory Course in Plant Physiology*, both devoted specially to simple experimenting in plant physiology. MacDougal's *Practical Text-Book in Plant Physiology*, a combined laboratory guide and text-book. Green's *Introduction to Vegetable Physiology* and Noll's *Physiology* in Strasburger's text-book are good modern reading books. Moor's translation of Detmer's *Practical Plant Physiology*, a standard work of reference for laboratory directions. Darwin and Acton's *Practical Physiology of Plants*, a valuable reference book. Ewart's translation of Pfeffer's *The Physiology of Plants*, the standard work for reference by the greatest living master of the subject.—W. F. GANONG, *School Science*, 2: 8.



## An Acid-Proof Table Top.

Three or four years ago the writer saw in a pharmaceutical journal (Merck's Report) a formula for a black finish for table tops. The article did not give the author's name nor the original source of the formula, but stated that the method was "used abroad." Further acknowledgment cannot, therefore, be made. The formula was as follows :

### 1.

Copper sulphate	-	-	-	-	-	-	-	1 part
Potassium chlorate	-	-	-	-	-	-	-	1 part
Water	-	-	-	-	-	-	-	8 parts

Boil until salts are dissolved.

### 2.

Aniline hydrochlorate	-	-	-	-	-	-	-	3 parts
Water	-	-	-	-	-	-	-	20 parts

Or if more readily procurable :

Aniline	-	-	-	-	-	-	-	6 parts
Hydrochloric acid	-	-	-	-	-	-	-	9 parts
Water	-	-	-	-	-	-	-	50 parts

By the use of a brush two coats of solution No. 1 are applied while hot. The second coat as soon as the first is dry. Then two coats of solution No. 2 and the wood allowed to dry thoroughly. Later a coat of raw linseed oil is to be applied, using a cloth instead of a brush in order to get a thinner coat of the oil.

The writer used this method upon some old laboratory tables which had been finished in the usual way, the wood having been filled, oiled, and varnished. After scraping off the varnish down to the wood, the solutions were applied, and the result was very satisfactory.

After some experimentation the formula was modified without materially affecting the cost and apparently increasing the resistance of the wood to the action of strong acids and alkalies. The modified formula follows :

### 1.

Iron sulphate	-	-	-	-	-	-	-	4 parts
Copper sulphate	-	-	-	-	-	-	-	4 parts
Potassium permanganate	-	-	-	-	-	-	-	8 parts
Water, q. s.	-	-	-	-	-	-	-	100 parts

### 2.

Aniline	-	-	-	-	-	-	-	12 parts
Hydrochloric acid	-	-	-	-	-	-	-	18 parts
Water, q. s.	-	-	-	-	-	-	-	100 parts

or

Aniline hydrochlorate	-	-	-	-	-	-	-	15 parts
Water, q. s.	-	-	-	-	-	-	-	100 parts

Solution 2 has not been changed except to arrange the parts per hundred.

The method of application is the same except that after solution No. 1 has dried the excess of the solution which has dried upon the surface of the wood is

thoroughly rubbed off before the application of solution No. 2. The black color does not appear at once, but usually requires a few hours before becoming ebony black. The linseed oil may be diluted with turpentine without disadvantage, and after a few applications the surface will take on a dull and not displeasing polish. The table tops are easily cleaned by washing with water or suds after a course of work is completed and the application of another coat of oil puts them in excellent order for another course of work.

Strong acids or alkalis when spilled, if soon wiped off, have scarcely a perceptible effect.

A slate of tile top is expensive not only in its original cost, but also as a destroyer of glassware. Wood tops when painted, oiled, or paraffined have objectionable features, the latter especially in warm weather. Old table tops after the paint or oil is scraped off down to the wood, take the above finish nearly as well as the new wood.

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## Cultures of *Empusa*.

It has been through a study of plant diseases, and especially in connection with those diseases caused by insects, that I have become interested in the diseases of insects caused by plants. A popular article<sup>1</sup>, containing a description of the principal diseases and the fungi which cause them, was prepared at the request of Prof. Bruner, Nebraska state entomologist.

When the grasshopper season opened in 1902, and the experiments with the South African locust fungus were resumed, it became my good fortune to be asked by Prof. Bruner to prepare the cultures of the fungus which were to be sent out into different parts of the state. Somewhat later, he asked me to spend a number of days in the field where the fungus was being used, and to report the results of my investigations as to the real worth of the South African locust fungus. My conclusions as to the value of the fungus for killing grasshoppers were all on the negative side. There were plenty of dead grasshoppers, but they had been killed by a "grub," the larva of a fly (*Sarcophaga*) similar in appearance to the common house fly.

However, there are fungi that kill grasshoppers in large numbers, one of them being an *Empusa*. Judging from the descriptions and illustrations in the South African and European journals of the positions that the grasshoppers are said to take at death, when dying from the effects of the South African locust fungus, I began to suspect that the real cause was not that fungus at all, but an *Empusa* instead.

It is no uncommon sight to see the weeds, grasses and alfalfa literally covered with dead grasshoppers during the latter part of the summer and in the autumn. Specimens of alfalfa were sent in from various parts of the state where the grasshoppers were said to be "dying by the millions," that were almost a

<sup>1</sup>Some Plants Which Live Upon and In Insects. Ann. Rept. Nebr. State Bd. Agr. 1901, p. 131.

mass of dead grasshoppers they were so thick, but they had not died from the effects of the South African locust fungus, as it had been supposed in a number of cases, but from the effects of "grubs," or an *Empusa*, or both.

Hairy caterpillars, larvæ of species of *Spilosoma*, are also found on the same plants with the grasshoppers. Upon finding some of the dead grasshoppers and caterpillars, an examination was made in order to determine the cause of death. It was readily ascertained that they had died from the effects of an *Empusa*. In a number of instances I have found a *Mucor*, very similar to the South African fungus which is a *Mucor*, on dead grasshoppers, caterpillars and flies.

The species of *Empusa* under consideration compares very favorably with the following description taken from Thaxter's<sup>1</sup> valuable monograph of the genus:

EMPUSA GRYLLI (Fresenius).

*Conidia* ovoid to pear-shaped, with a broad papillate base and evenly rounded apex; 30–40  $\mu$  x 25–36  $\mu$ ; hyaline and containing one or more large fat globules. *Conidiophores* simple, coalescing externally when growing luxuriantly, and arising from rounded irregular hyphal bodies, with or without subsequent branching. *Cystidia* wanting. *Secondary conidia* of one kind, like the primary. *Resting spores* spherical, colorless; 30–45  $\mu$  in diameter; produced terminally or laterally from hyphæ, directly within or by budding from hyphal bodies; or by a pseudo-conjugation between two divisions of a single hyphal body. Host attached to substratum by the contraction of its legs.

Both the grasshoppers and caterpillars grasped the plants upon which they were found so tightly, that their legs were sometimes broken off in attempting to remove them.

There was no indication of the presence of a fungus on any of the grasshoppers when they were found in the field, and, for the most part, on but a few of the caterpillars during dry weather. When it was damp, as after a heavy dew or shower, caterpillars were occasionally found which were covered with a mass of fungous hyphæ, the color of which was modified to some extent by the color of the hairs of the caterpillar mixed with the hyphæ. The hairs were covered with what proved on examination to be whitish, granular masses of sticky spores that held the hairs together in bunches.

When the grasshoppers and caterpillars, which had not been dead too long, were put into a Petri dish with a few fresh leaves or a little water, where they could be kept in a moist atmosphere, the hyphæ of the fungus soon began to protrude through the segments of the body, finally covering it so that the body, in the case of the caterpillars, appeared as if swollen to nearly twice the normal size. If the hairs were removed before the caterpillars were put into the Petri dishes, the swelling was much more pronounced. Better success was had in developing the fungus on caterpillars than on grasshoppers. Many of the grasshoppers had decayed to such an extent when they were found, that the fungus could not be made to develop, while others were filled with resting spores, or

<sup>1</sup> Thaxter, Roland. The Entomophthoræ of the United States. Mem. Boston Soc. Nat. Hist., Vol. iv, 1888.

both resting spores and "grubs." On account of the better condition of the fungus in the caterpillars, they have been used much more than the grasshoppers.

In a few hours after the caterpillars had been placed in the Petri dishes the fungus began to show between the segments of the body, conidia being thrown off with considerable force in all directions. The conidia which are sticky adhered to the sides and top of the dishes, giving them a frosted appearance. Moisture condensed on the inside and caused the spores to germinate within a comparatively few minutes. It is probable that infection occurs by the sticky conidia becoming attached to an insect, although I have not actually observed it. Toward evening grasshoppers climb to the tops of the plants on which they have been feeding. If there were dead ones there, conidia might be developing and be thrown on to the live ones. This might happen any night when the air was damp. If the body of the grasshopper was slightly moist where the conidium was attached, it would germinate at once and in all probability the hypha of germination would enter the body.

Hyphæ which were protruding from the bodies of caterpillars were removed and drawings<sup>1</sup> made from them. The hyphæ were for the most part simple, though there was occasionally one that was branched (Fig. 1), and divided by septa into cells of different lengths. Most of the cells were empty except the terminal one, which was considerably enlarged and filled with a vacuolated mass of protoplasm (Figs. 1-3, 8). The terminal cell functions as a basidium (Figs. 2, 3, 8). Conidia in different stages of development were attached to the basidia (Figs. 2*a*, 3*a*, 8*a*). There were also basidia from which the conidia had become detached (Figs. 4, 8*b*), open at the top, and presenting the appearance of a slender vase with a short neck just below the more or less ragged mouth.

On examining the body cavities of the grasshoppers and caterpillars before the hyphæ had begun to show on the outside, they were found to be packed with rounded, irregular-shaped bodies (hyphal bodies) (Figs. 15, 16) and empty filaments or else hyphal bodies mixed with masses of filaments containing protoplasm and lying parallel to one another. The hyphal bodies were at times considerably elongated and arranged in the same way. The empty filaments might have been mistaken for pieces of detached hyphæ from the hyphal bodies, had it not been for a few in which the hyphal body appeared as if lying in the filament after the manner of chlamydospores. In some cases the hyphal bodies were rounded (Fig. 16) and resembled spores, others were irregular in shape and with one or more projections (Fig. 15).

The hyphal bodies seem to be a partial resting stage of the fungus, for when the insects were placed in the moist chamber the hyphal bodies sent out hyphæ which pushed through the segments of the body and finally bore conidia. None of the hyphal bodies were found in the alimentary canal, but were in other parts of the body, even in the grasshoppers' legs. Just what these hyphal bodies are, it is difficult to say, but they appear to be the result of the breaking up of the mycelium of the fungus after it has used up the greater part of the available nutriment contained in the body cavity. The finding of interstitial bodies in the

<sup>1</sup> All of the drawings have been made by means of the camera lucida and microscope, No. 4 ocular and No. 3, 5 or 8 objective.



filaments would tend to prove it (Fig. 9). The hyphal bodies are often found attached to one another in considerable masses, each with a distinct cell wall or as a "bud," the separation not being complete. Both hyphal bodies and resting spores were found in the same grasshopper. In all probability, if the hyphal bodies do not germinate and produce conidia on the outside of the body, they gradually become spherical, put on an extra cell wall and become resting spores. As yet I have found no resting spores in caterpillars corresponding exactly to those found in grasshoppers. There were some so much like resting spores in size and shape that it was a difficult matter to tell which was which when they were lying side by side. Both are spherical, had two heavy cell walls, and the internal structure was similar (Figs. 13, 14). The one essential difference was that the cell wall of those obtained from the grasshoppers was slightly colored. The thick walled bodies (Figs. 9-13) in the caterpillars were not formed until after they had been kept in the laboratory for over four months; they were of various sizes and shapes, from spherical to cylindrical and irregularly curved; they occurred singly in filaments (Fig. 9) and in masses loosely joined together, separation having apparently taken place by either budding or fission (Fig. 12). Whether these irregular shaped bodies will in time become spherical remains to be determined, also whether they still retain their vitality and are capable of germination. I have not yet been able to germinate the resting spores from grasshoppers, but I feel confident that it can be done by exercising a little ingenuity and patience. When it is time for them to germinate they will undoubtedly do so readily. Considerable difficulty has sometimes been experienced in attempting to germinate certain seeds during the autumn and winter, yet they germinated when it was time for them to do so naturally. A "resting spore" may not be resting, but on the other hand doing something—maturing—and will germinate only after it has matured and under the proper conditions. The hyphal bodies and conidia when fresh germinated after being in water for a short time.

#### CULTURES.

*Cultures from Hyphal Bodies.* When I found out that the conidia and hyphal bodies would begin to germinate and develop hypha almost as soon as put in water, I began to wonder if it might not be possible to grow this *Empusa* on the same culture media that I had been growing the South African *Mucor*, although I was well aware that *Empusa* is considered as an obligatory parasite, parasitic upon certain species of insects.

Nevertheless, hyphal bodies were removed from both grasshoppers and caterpillars and cultures made from them in "poured plates" of bouillon-agar and by "planting" them in plates of agar by means of an inoculating needle. It was found to be a difficult matter to obtain pure cultures by either method. The hyphal bodies do not separate readily; they were already contaminated when removed from the body cavity with the filaments of other fungi and bacteria. The contaminations were removed in part by washing. The amount of contamination varied considerably in different insects; those that had recently died of course containing much less than those that had been dead for some time.



PLATE I.

FIG. 1.—Branched hypha from caterpillar. 2, 3.—Basidia with conidia from caterpillar; *a*, conidia forming. 4.—Basidium from caterpillar from which the conidium has been thrown off; *a*, place of attachment to the conidium. 5–7.—Conidia from caterpillar; *a*, papillate portion which extended into the basidium. 8.—Mass of basidia from caterpillar; *a*, conidium; *b*, empty basidia from which conidia have been thrown off; *c*, place of attachment to conidium; *d*, basidia before conidia have formed. 9–13.—“Resting bodies” from caterpillar. 14.—Resting spore from grasshopper. 15.—Hyphal bodies from caterpillar. 16.—Hyphal bodies from grasshopper. 17–23.—Hyphae developing from hyphal bodies from grasshoppers. 24–26.—Formation of conidia in agar culture of hyphal bodies from caterpillars. 27.—Discharge of conidium (*a*) in agar culture of hyphal body from caterpillar; *b*, place of attachment to conidium.

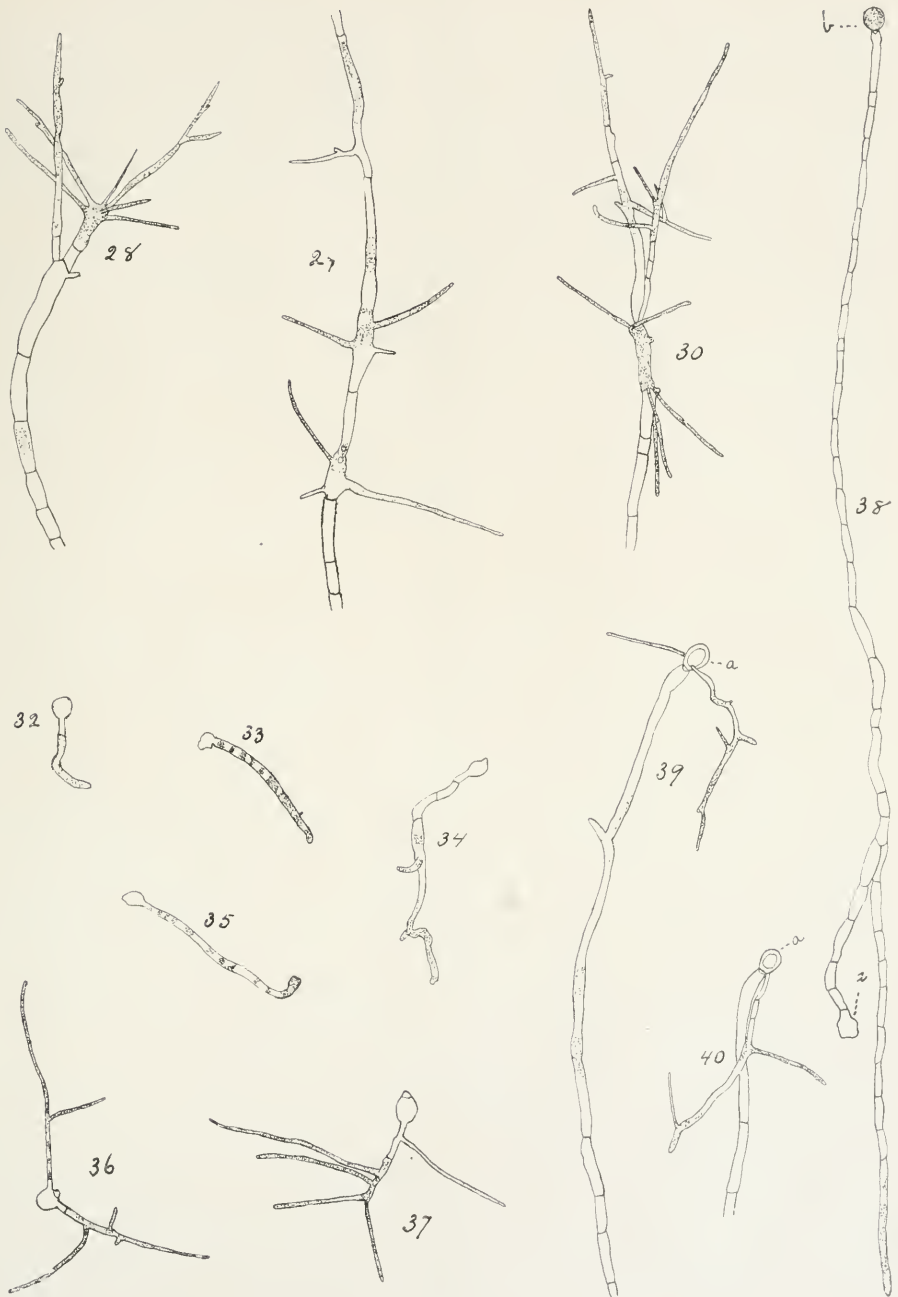


PLATE II.

FIGS. 28, 30.—Sections of mycelium in agar cultures from hyphal bodies from caterpillars.  
 32–35.—Conidia from caterpillar germinating. 36, 37.—Germinating conidia on agar from  
 hyphal bodies from caterpillars. 38.—Development of the fungus in agar from a conidium  
 (a), produced from a hyphal body from a caterpillar, to the formation of a new conidium (b).  
 39, 40.—Germinating conidia (a) in agar, produced from hyphal bodies from caterpillars.

Pure cultures were obtained, however, after something like two hundred trials and nearly as many failures.

The hyphal bodies began to send out hyphæ (Figs. 17-19) into the agar shortly after they were placed in it, and, although the medium was evidently not exactly suited to the best development of the fungus, the hyphæ continued to grow until prevented by bacteria and other fungi. One pure culture continued to show signs of growth for 26 days.

A caterpillar-agar was prepared, but it did not give as good results as the bouillon-agar. The reason for not giving better results was attributed to the large amount of vegetable matter contained in the caterpillars which were used, so that the medium was a vegetable-agar containing a small amount of a decoction of caterpillars rather than a caterpillar-agar. Growth on this medium was very rapid for a while, and I entertained the most sanguine hopes that I had hit upon just the medium suited to the fungus, but I was sorely mistaken, for growth ceased about as suddenly as it began, probably when the reserve material stored in the hyphal bodies and conidia had been used up. It therefore remains a question whether the fungus made use of any of the medium except the water which it contained.

On germinating, the contents of a hyphal body extended out into the hypha, leaving the hyphal body empty (Figs. 17-19). As the hypha continued to develop septa were formed behind the constantly receding mass of protoplasm (Figs. 20-23). The movement of the protoplasm in the hyphæ resembled that of the *Mucoraceæ* so much that the filaments of the *Empusa* might have been mistaken for one of them.

Some of the hyphæ on reaching the surface of the agar formed basidia. A "bud" appeared at the end of the basidium, gradually becoming larger and larger until a septum separated it from the basidium as an egg-shaped conidium (Figs. 2a, 3a, 8a, 24a-26a). One peculiarity about the attachment of the conidium to the basidium was that instead of being attached to the conidium, a papillate portion of the conidium extended down into the basidium (Figs. 3a, 8a, 26a), so that when the conidium became detached a scar marked the place of attachment (Figs. 5a, 7a), leaving the end of the basidium open and with a ragged edge (Figs. 4a, 8c, 27b). The manner of attachment of the conidium to the basidium probably has something to do with the ejection of the conidium, for when the conidium is mature it is thrown some distance from the basidium (Fig. 27a). Conidia formed in the cultures, falling upon the moist agar germinated (Figs. 36, 37, 38a), and the hypha from one (Fig. 38a) was kept growing until another conidium (Fig. 38b) was produced, thus completing the life history from a hyphal body to a conidium of the second generation. Some basidia produced conidia in the agar without reaching the surface. The conidia of course could not be thrown off and germinated where they were formed (Figs. 39a, 40a).

There seemed to be an attempt on the part of certain cells interposed with the other cells of the hyphæ to produce hyphal bodies or chlamydospores. (These hyphæ were from hyphal bodies and not from conidia.) The cells which were filled with protoplasm remained apparently inactive for a number of days,



then began to put out branched filaments in various directions, after the manner of germinating hyphal bodies or conidia (Figs. 28-30). The cause for this second growth cannot be explained unless it be by some change in the amount of moisture in the air. Had not this growth occurred, there is a possibility that resting bodies would have been formed the same as was found in the caterpillars that had been kept for some time.

*Cultures from Conidia.* Since I had had so much difficulty in obtaining pure cultures from hyphal bodies, I thought by resorting to the conidia from the caterpillars that a much larger percentage of pure cultures could be obtained. Caterpillars were placed in sterile Petri dishes, one caterpillar to a dish, with a few drops of water at either side of the caterpillar, but not touching it. When the conidia began to be thrown upon the cover they were removed with a sterile needle and cultures made from them. Even with these precautions bacteria, yeast and fungous spores were found to be attached to many of the conidia. They probably became attached to the surface of the basidia from the outside of the caterpillar, or were carried from the inside of the body when the hyphæ broke through the segments. Something like three hundred plates were made from conidia obtained in this way, and not over one per cent. of them was pure.

The conidia germinated readily, and the hyphæ grew rapidly for a few days (Figs. 32-35), but they did not give as good results as the conidia which were produced from the hyphal bodies grown in agar. There is a possibility that the fungus in the latter case had begun to adapt itself to the medium and become semi-saprophytic. The growth of the hyphæ from the conidia was essentially the same as those from the hyphal bodies. A conidium produced one or more hyphæ, the contents of the conidium passing into the hypha, leaving the cell wall of the conidium empty. Septa were formed in some of the hyphæ in about fifteen hours after the conidia germinated. The hyphæ from the conidia obtained directly from the caterpillars were for the most part simple, and but one hypha from a conidium (Figs. 32-35), while those from the hyphal bodies grown in agar were either simple or branched, and with one or more hyphæ from a conidium (Figs. 36, 37, 38), indicating that a mycelium may be formed in the body of the host. The difference in the amount of branching can probably be attributed to the difference in the age and not to any essential difference in the conidia themselves.

It will be necessary to experiment with media of different compositions before one is obtained that is suited to the best development of the fungus, or else the fungus must be able to gradually adapt itself to some standard medium before it can be grown with anything like success and in any considerable quantity, in a medium more closely resembling the contents of the body cavity of insects, one containing the essentials of muscle and fat. A bouillon-glycerin medium is being tried for cultures of *Empusa* from the house fly. Sufficient work has not yet been done to test it thoroughly.

Now that it has been demonstrated that *Empusa* can be grown artificially, the next thing to determine will be what the proper medium is and the conditions under which it grows best. No economic importance can be attached to it for the destruction of grasshoppers or other noxious insects, more than what

it brings about naturally in the field, unless it can be grown in quantities, and inoculations be made by any person of ordinary intelligence and good common sense. The grain field is not a place suited for the carrying on of experiments in the culture and inoculation with fungi by the methods that can be employed in a bacteriological laboratory. The experiments that have been carried on, not only in the United States, but elsewhere in attempting to destroy chinch bugs and grasshoppers by inoculating them with cultures of different fungi, have been only partially successful. It has not been a difficult matter to grow the fungi in sufficient quantity with which to make the inoculations, but the uncertainty of atmospheric conditions suitable for the germination of the spores and the entrance of the hyphæ into the bodies of the insects inoculated has been the one serious disadvantage over which there is no control.

As yet I have not tried to inoculate either grasshoppers or caterpillars from pure cultures of *Empusa*. I have only made a beginning in growing it artificially. In all probability if it can be grown in quantity it will be of no more value in exterminating insects than *Sporotrichum* and the South African *Mucor*. But while little may be expected from an economic standpoint, there is something to be gained from a study of its cultural characters both morphologically and taxonomically. So far as I am aware it has not been grown before in an artificial culture medium; if it has, I shall be duly grateful to anyone who will inform me concerning the methods employed and with what success it was grown.

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## A Method of Demonstrating Involuntary Muscle Fibres.

The following method of demonstrating involuntary muscle fibres will sometimes be found convenient, and will give very fair results. The method will be found especially useful in cases where the preparation is needed in a hurry, and there is not time to wait for the fresh tissue to macerate.

A celloidin section of some organ containing involuntary muscle, i. e., the small intestine, is taken from the laboratory supply, and is stained deeply with some double stain, i. e., hæmatoxylin and eosin. The section is then put into a flat dish or watch-glass of absolute alcohol or synthol; the celloidin is thus quickly dissolved and the muscular layer (in the case of a cross section of the intestine, the circular layer) may be easily separated from the other tissues, and, with sharp teasing needles may be very finely teased. This teased material is then dropped into a narrow bottle containing xylol or some other clearing fluid, the narrow bottle being used so that the excess of clearing fluid may be easily removed and the muscle fibres be thus transferred to a slide, with a minimum amount of clearing fluid. A drop of balsam and a cover-glass complete the operation, and though there will probably not be many fibres that are entirely separate from the others, a very good idea of the size and shape of the fibres and their nuclei may be obtained.

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## The Museum.

### IV. INTERIOR PLAN.

The interior arrangement of halls, corridors, lecture rooms, etc., admits of variation determined by character of exhibits and building, and is naturally influenced by particular aims in the management, or original purpose of the museum. For museums generally, however, some useful, fixed plan and relation of parts can be insisted on.

First, a well lighted basement extending under the whole building is indispensable. This basement contains heating and ventilating and power plants, and usually the direct supply from a basement of heat and power will be found more convenient and economical than the erection of an outer building for both.

Into such a comfortably and carefully divided basement, all incoming freight may be delivered, recorded, unpacked, and prepared for treatment in the laboratories, or made ready for direct introduction on the exhibition floors. The avoidance of a great deal of dust and dirt is thus made possible, and a general inventory secured of all receipts and shipments.

Material from the departments should be forwarded from the basement, and here the store rooms, carpenters' shops, and, if the position is a dry one, the duplicate rooms be maintained. An allotment of one or more rooms to each department for the rougher preparation of specimens, mounting, and cleaning can be located in the basement.

It seems feasible to place in the basement, at extremities where area-ways can be constructed for their full illumination, the rooms of taxidermy, modelling rooms, the disinfecting and poisoning apparatus, dessicating rooms for skins, bones, bleaching baths, etc., and even the boxes for study collections. In this way the noxious, unhealthy, and intolerable suffusion of odors (accompanied also by destructive gases) through a museum can be sensibly diminished or entirely banished.

If the basement is thus serviceable for the installation of the preparatory mechanical and shipping work of the museum, the top floor should be reserved for offices, laboratories, and administrative chambers, unless, as in art museums, the top floor is cut up into galleries. Art museums, from their diffuse structure, and their freedom from the annoying features of organic preparations and their preservation, can readily devote dry, airy, and lighted basements to the use of general offices as well as of freight and storage rooms.

This provision of rooms for labor in the bottom, and rooms for research and direction in the top of the museum will be found helpful. Frequently, by reason of the towers used to diversify or assist in the construction of museums, small useful office rooms are formed on every story. These, of course, can be used for curators, but it seems preferable to make some other use of them, keeping the working staff segregated at the top or bottom of the building. It facilitates intercourse, communication, etc., and is a better system for the public and special visitors.





each other by corridors (Fig. 20), and even then not to allow a dimension less than 50 feet square. The towers and angles of a museum frequently furnish restricted areas where such individual displays can be made, but the practice is pernicious. *The large hall should be steadily adhered to* as the exhibition space of science museum, and rooms, apartments, cells, galleries, corridor exhibits, and small spaces resolutely frowned down. Large halls admit of rearrangement, readjustment, replacement; they furnish the best opportunities for experiment; they accommodate the public better; they are more easily cleaned and kept clean; they are almost invariably better lighted; they are more healthy and

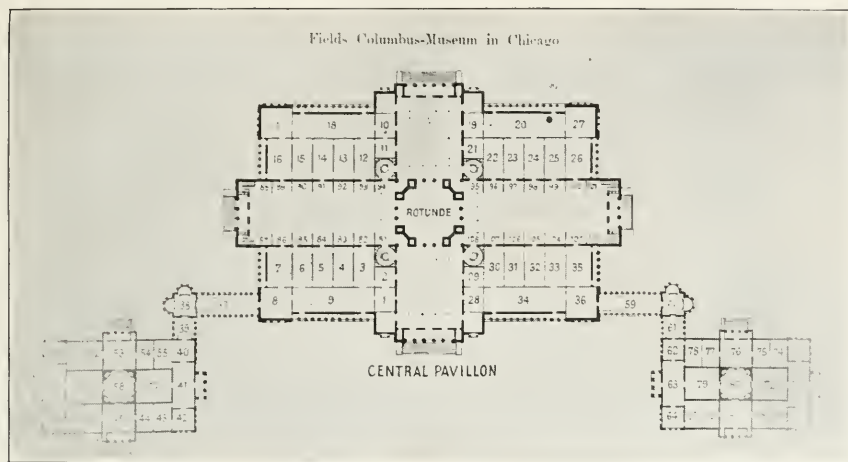


FIG. 20.—Plan of Field Museum, Chicago.

more cheerful; they preserve a unity of design in the interior of the museum, and they allow the greatest prolixity and variation of arrangement in the exhibits; cases of all sizes, and, if it is desired, all forms, can be accommodated in them, and every problem of museum installation is more quickly and to greater satisfaction solved in them than in restricted, cramped, irregular, or broken spaces. Had such large halls been provided for the Free Public Museum in Liverpool the recent report (1901) upon the unsatisfactory condition of the Lord Derby collections would have been less despondent.

L. P. GRATACAP.

American Museum of Natural History.

**DISSECTING NEEDLES.**—A dissecting needle of suitable shape is often of great convenience. If it is desired to bend it, it should be heated to a dull red and allowed to cool gradually; it can then be bent into any shape, and if desired an edge can be ground or filed. To reharden, heat as before to blood-red heat and plunge into cold water. In this condition it will be found too hard and liable to break easily. It should be rubbed bright on fine emery paper, then held in a spirit lamp until it assumes a pale straw color and again dipped into cold water. The correct temper for use will then be obtained.—*Knowledge*, 26: 209.

## The Technique of Biological Projection and Anesthesia of Animals.

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### XII. THE ANESTHESIA OF ANIMALS.—Continued.

**MOLLUSCA: *Snails*.**—Specimens with transparent shells may be used for the study of the heart beat and various morphological details, while both small and medium sized snails may be employed when ciliary action is to be looked for on the surface of the tentacles and foot.

Place the animals in a watch-glass in water enough to cover them and add one-half its volume of one per cent. chloretone solution. Some species require an increased amount of the anesthetic up to equal volumes with the water.

***Polyzoa*.**—The sensitiveness of these delicate and beautiful animals makes their careful study in the live state under hand lens or compound microscope a difficult task, unless some anesthetic suited to their peculiarities is employed. Chloretone has served admirably for the species thus far tested.

Place one or more specimens in a watch-glass with water enough to cover them and allow them to stand quietly until the tentacles are expanded. Add one per cent. chloretone solution drop by drop from a pipette, watching the effect until the desired degree of anesthesia has been attained. If at any time too much chloretone has been added, water may be dropped into the watch-glass to dilute the solution. The tentacles, lophophore, and general morphological details require a less complete degree of anesthesia for their study than do the cilia which thickly clothe the tentacles. To study the various degrees of activity of the cilia from



FIG. 7.—Optical Section of Head and Prothorax of Nymph of Dragon Fly. Photographed from screen, as studied by a class, to demonstrate the usefulness of projection methods in teaching the anatomy of live animals and the value of anesthesia.

the normal rapid motion, during which the individual cilia are not visible, through the stage in which the movements are slower and the cilia appear to act in groups, to the final stage of activity which is characterized by slow and irregular motions of each cilium, it is necessary to add chloretone solution drop by drop while the animals are under the microscope. Before the last stage is reached the tentacles become distorted by an angular bend.

**VERTEBRATA: *Goldfish*.**—Small goldfish, *i. e.*, from two to three inches long, are among the most desirable, if not the best, vertebrates in which to

observe the circulation of the blood and the wandering of the white blood corpuscles; for they are easily kept alive in the laboratory, are quickly prepared for study, and the arterial and venous trunks in the caudal and other fins, being approximately parallel, are more apt to be found in the same field and focal plane of the objective than are the corresponding parts of the web of a frog's foot. Moreover, the capillaries are more easily traced from artery to vein than in the frog's web. In addition, the fin-rays and their articulations and also the lateral line are readily seen.

To anesthetize goldfish for the purpose of sketching them, studying them under hand lens or compound microscope, proceed as follows: Place the fish, one or more as needed, in a tray with water enough to cover them and add to the water one-twentieth its volume of one per cent. chlorotone solution. Active swimming soon ceases, the movements of the jaws, which are at first rapid, gradually decrease, the movements of the pectoral and ventral fins become irregular and cease, and finally the fish turns on its side and appears to be dead. It is now in the most favorable condition for sketching and for careful morphological study. To prepare it for study under the compound microscope place it on a piece of clear glass an inch or two longer and wider than the fish, spread the caudal fin, moisten the body of the fish with enough of the anesthetizing solution to form a little pool around it on the glass, and transfer the glass plate and fish to the stage of a microscope, the stage being approximately horizontal. The fish does not need to be tied or pinned down. If the study is to continue for a half hour or more, place a piece of filter paper or cloth moistened with the anesthetizing solution over the entire body in front of the tail so as to prevent the epidermis and scales from becoming dry. A thin cover-glass may be laid on the caudal fin for the same purpose. Focus on the caudal fin and trace the blood stream from arteries through arterioles, capillaries, venules and veins. Using a quarter inch, or higher power, objective, examine the thin end of the caudal fin for wandering cells in the connective-tissue between the capillaries. The leucocytes here appear flattened, circular or irregular in outline, and very granular. Examine the irregular shaped ones and note the slow ameboid movements which accompany the projection or contraction of their pseudopodia. It is possible to make out the motion of individual granules in the moving leucocytes. By careful focussing and lighting the outlines of the epidermal cells are brought into view.



FIG. 8.—Tracheal Gills of Nymph of Dragon Fly. Photographed from screen.

To study the pits and tubes of the lateral line, the fish is mounted as above described and the microscope is so arranged that direct sunlight, or a strong artificial light, will illuminate the part on which the objective is focussed. The circulation of the blood in the capillaries at the bottom of the pits and in the skin overlaying the scales is easily seen with a half-inch objective.

The length of time during which a fish will continue to live and exhibit a vigorous circulation when anesthetized with chloretone and mounted for study in this simple manner, depends upon its vitality and the strength of the anesthetic. The proportions of water and chloretone solution suggested above give a solution of minimum strength and it may need to be supplemented occasionally with a drop of one per cent. chloretone placed over the gill-slit so that it will run down over the gill-filaments. This should be done whenever the least motion of the fish is noticed as it lies on the glass plate. A goldfish about two and three-quarters inches long, treated in this way, was studied for over two hours and the circulation continued to be good up to the time it was returned to the aquarium, where it revived. Amœboid movement of the white blood corpuscles and the movement of the granules in the corpuscles was clearly seen near the close of the period of observation.

Repeated use of this anesthetic at frequent intervals is not usually fatal to goldfish. For example, ten fish were anesthetized in one tray, then distributed to a class whose members made careful drawings, consuming about forty-five minutes, after this the fish were returned to clear water for fifty minutes, then again anesthetized for the same length of time, and all revived within a few hours after being placed in clear water. Their recovery from the anesthetic seems to be hastened by artificial respiration, which is easily accomplished by injecting several pipettesful of clear water into the mouth so that it escapes through the gill-clefts, or by repeatedly lifting the operculum with a blunt needle while the fish is loosely held under water.

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## The History of the Microtome.

### II. THE EARLIEST TYPES—Continued.

The third initial form of microtome was that devised by Rivet, a French botanist, which was described in the *Annales des Sciences Naturelles*. In Rivet's microtome the feeding of the object was accomplished by shoving it up an inclined plane and not by means of a screw, a principle which is preserved in the Thoma-Jung, or so-called Heidelberg microtome, which is still so much used. Rivet's instrument was made of wood and proved, for botanical purposes, a useful instrument. It was made by Vêrick, the world known manufacturer of microscopes in Paris. I have never seen one of these instruments. It was, however, copied in Germany, with the improvement that it was made of brass instead of wood, permitting, of course, a very much more exact construction.

In 1870 Alexander Brandt, who afterwards became a distinguished zoölogist,



was working in the zoölogical laboratory of Prof. Leuckart at Leipzig, where they were then using the Rivet microtome. The unsatisfactory results obtained with the wooden model led Dr. Brandt to have made by Leyser, the mechanician of the university, the microtome which was long and widely known by the name of Leyser, and to which I have referred above. Of this instrument I reproduce the original figure, published by Brandt in Vol. VII of the *Archiv für mikroskopische Anatomie*, page 176. I still possess one of these early instruments, which has seen a great deal of use. Its construction constituted a great step forward. The essential addition by the Rivet type to what we had before had was the substitution of the mechanical motion of the knife for the free-hand motion, so that both object and knife were moved mechanically and therefore with comparative precision. Perhaps the principal reason why this microtome gained favor so rapidly is to be found in the fact that just before its introduction the embedding of objects in paraffine had been introduced, and the microtome was used chiefly for paraffine sections. The microtome itself may be described as follows:

It consists of a median vertical plate, on either side of which are two ways. The way on the left hand side is inclined, being higher at one end of the instrument than the other. On this way there runs a sledge that carries the object holder which, as Dr. Brandt states, was made on the pattern of an American patent clothes pin. This holder need not, I think, be further described. As the sledge with the object holder and object would move along the way, it would of course rise, the usual pitch being at the rate of 1 to 20. A scale on the microtome enabled one to read off the thickness of the sections. The sledge would move forward by hand and of course the position had each time to be read off in order to make sure that the thickness of the section was that desired. The way on the opposite side at the back of the figure was horizontal and upon it was a second sledge, which carried the knife, the knife end being held in place by means of a screw and clamp. In those days the knife was always placed in an oblique position, even for paraffine cutting, and this is indicated in the illustration. It was not until later that it was discovered that good sections of paraffine can be made with the knife at right angles to the line of pull or draw. From the Leyser microtome have sprung numerous modifications, to some of which I shall have occasion to refer later.

The next important step in the development of the microtome was a combination of the Ranvier type with the mechanical movement of the knife. The object holder in this type, of which the Schanze microtome is perhaps the best illustration, is attached to a sledge or carriage which moves in vertical ways and is raised in these ways by means of a micrometer screw with a very large head.

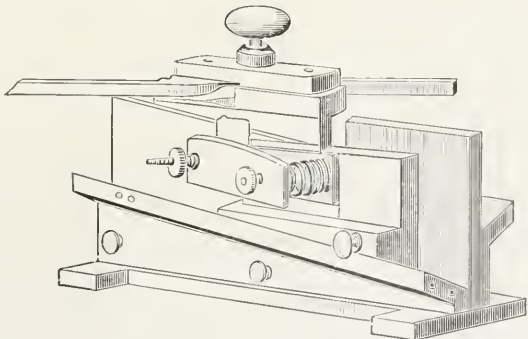


FIG. 4.—Leyser-Brandt Microtome.

The knife is moved in the same manner as in the Leyser microtome just described. The following description refers to one of the earlier instruments of this type. Schanze is an instrument maker in Leipzig, and copied in part the system of construction used by Leyser, for he employed a horizontal way along which the carriage moves, which carries the knife. The large head of the micrometer screw was graduated and an index was placed so that one could easily turn the head of the screw any desired distance and obtain the desired raising of the object and thickness of section. But a still more important improvement, which appears in the Schanze instrument, is the introduction of the adjustable object holder, so devised that it can be turned around two horizontal axes and, after turning, be clamped securely in any position. By this device the plane of the section can be adjusted and any desired angle secured. In addition the actual object holder was fastened upon a vertical rod which fitted into a vertical hole so that the object holder could be revolved around the vertical axis. After the desired position is obtained, the rod can be fastened by means of a clamping screw. We have in this object holder all of the adjustments which are to be found in the best modern patterns, though some of the mechanical details in the construction of later object holders are better than in the original Schanze pattern. The most important improvements in the object holder are represented by those which are embodied in what is familiarly known as the Naples holder, which is now used in many of the best microtomes.

To recapitulate, we find that the mechanical movement of the object to be cut was first introduced in an available manner by His and Ranvier; that the mechanical movement of the knife was first employed by Rivet, but the successful employment of metal in instruments with a mechanical movement of both the object and the knife was first adopted for the instruments made by Leyser, under the direction of Brandt; and, finally, that in the microtome made by Schanze we have an object holder with adjustment around two horizontal axes and revolution around a vertical axis, also a combination of the mechanical movement by means of the micrometer screw with mechanical movement of the knife. The three essential things which appear as the product of these early inventions are, therefore, mechanical movement of the object, mechanical movement of the knife, and mechanical adjustment of the plane of the section. These three fundamental requirements must be met by every microtome which aims to fulfill our present demands.

We thus see that after several preliminary inventions have been introduced, there come four instruments: that of Ranvier, of Rivet, of Leyser, and of Schanze, which together represent all the essential features which have been followed in the construction of microtomes, until we get to the introduction of the automatic instruments which involve new principles. All further accounts, therefore, must first review the history of the modifications of, and the development of the accessories to, the types of microtome indicated in the preceding descriptions. After that we shall recur to the history of the automatic microtomes and the modifications which have gradually occurred in them.

## A Review of the Methods of Staining Blood.

### VI.

#### *D. Neutral Stains—Continued.*

##### **5. The Development of the Neutral Eosin-Methylen Blue Method of Staining Blood.**

—For years the neutral triple stain and its modifications have been the standard stains for the blood. They produce a considerable differentiation of the complex elements of the blood, but they have weak basic properties and do not stain the basophile granules of the red cells, the granules of the mast cells, or the malarial parasites and bacteria when present in the blood. Recently, however, students of the blood have been devoting their attention to staining with eosin and methylen blue. We have in these two dyes the most powerful and at the same time the most precise acid and basic stains that are known for staining the blood, and hematologists have succeeded in combining the two into a neutral stain with which we can obtain a complete differential staining of the blood.

As we have already seen in the section on double staining with acid and basic stains, several hematologists, including Chenzinsky, Plehn, Aldehoff, Lavran, Gabritschewsky, Canon and Pielicke, and Mannaberg, have employed eosin and methylen blue, either successively or in combination, for staining the acidophile and basophile elements of the blood. But Romanowsky (1891) appears to be the first to obtain a neutral stain from the combination of these two dyes.

*Romanowsky's* stock solutions consisted of:

- (1) Saturated watery solution of methylen blue.
- (2) 1 per cent. watery solution of eosin.

These two solutions can be kept for a long time; the older the methylen blue solution the better its action. Dry preparations heated for 30 minutes at 105° to 110°C. are floated on the surface of a mixture of 1 part of the filtered methylen blue solution to two parts of the filtered eosin solution, contained in a watch glass, for from 2 to 3 hours, the preparations being covered by another inverted watch glass, and the whole by a bell jar moistened on the inside. The preparations are then washed in water, or, if overstained, first in alcohol.

When the two stains are mixed a heavy precipitate falls which should be used together with the solution. Romanowsky assumes that a third neutral color is formed, for which the nuclear network of the malarial parasites shows the greatest affinity. The malarial parasites are stained Prussian blue, the nuclear chromatin carmin violet. Presumably the basophile, acidophile and neutrophile elements of the blood are also stained.

Romanowsky's method of staining has, however, given variable and unsatisfactory results and has been superseded by improved methods. These improvements have consisted first in the use of a solvent for the neutral dye. Ehrlich found that the neutral precipitates from the mixtures of acid and basic dyes were at most but slightly soluble in the fluid of the mixture. He obtained a solution of the neutral dye by the addition of an excess of the acid dye, but consequently obtained a weak basic staining. Those who have worked with the Romanowsky

stain have employed various reagents, as soda, alcohol, acetic acid, etc., for dissolving the eosin-methylen blue precipitate. Secondly, there has been the production by various treatment of a polychrome condition of the methylen blue, whereby there is secured a greater differentiation in staining. And finally several hematologists have succeeded in isolating the active staining ingredient of the compound dye and applying it as a separate stain.

*Nocht* (1898) modified the Romanowsky stain as follows: To 2 or 3 drops of a 1 per cent. eosin solution diluted with 1 to 2 c. c. of water is added drop by drop a cool solution, consisting of 1 per cent. methylen blue and one-half per cent. soda which has stood several days in a thermostat at 50° to 60°C. until the eosin solution becomes more and more blue-red, and finally so dark that the original eosin solution can scarcely be recognized. On this mixture the blood preparations swim 5 to 10 minutes.

*Laveran* (1900) recommends the following eosin-methylen blue compound for staining malarial blood:

Solution No. 1. Silver oxid methylen blue (Borrel blue) is mixed with 150 c. c. of distilled water in a flask. Enough of the crystals are used to form a nearly saturated solution. When the dye is dissolved the flask is filled with a solution of soda and shaken, which causes a black precipitate of silver oxid. This is carefully washed and a saturated aqueous solution of medicinal methylen blue is poured over it and allowed to stand eight days, shaking occasionally.

Solution No. 2. An aqueous solution of eosin 1 to 1000.

Solution No. 3. A 5 per cent aqueous solution of tannin.

The stain is prepared just before using as follows:

Solution No. 1	-	-	-	-	-	1 c. c.
Solution No. 2	-	-	-	-	-	4 c. c.
Distilled water	-	-	-	-	-	6 c. c.

Each should be filtered before mixing.

Blood smears are fixed in alcohol 20 minutes and stained 5 to 10 minutes. When stained the preparation is treated with the tannin (Solution No. 3) for a minute. Nuclei are stained deep violet, red corpuscles pink, protoplasm of malaria parasites a pale blue, chromatin a violet-red.

*Laurent* (1900) studied the chemistry of the neutral stains and determined from their molecular weights the proper proportions of the two dyes to mix for a neutral stain. He used 1 per cent. solutions and to 1000 c. c. of the eosin solution was added 882.3 c. c. of the methylen blue solution. The mixture is allowed to stand at least 48 hours, when almost all of the neutral dye will have fallen as a precipitate. The mixture stands as a suspension of neutral dye in a watery fluid. This suspension remains good for 6 months, and will keep indefinitely if the solution is kept practically sterile after the fall of the precipitate by storing, after thorough shaking, in small, well-corked flasks.

For staining one takes 1 part of this well-shaken mixture to 24 parts of water in a reagent glass and brings it to a boil as quickly as possible over the flame of a Bunsen burner. As soon as the solution comes to a boil the reagent glass is cooled somewhat in water, and the preparation to be stained is placed in this still warm clear fluid. A too hot solution spoils the preparation. The staining



is sufficiently strong in half an hour, but the preparation may be left in the staining fluid 6 hours without injury. The preparation without washing is dried between filter paper, and then waved to and fro in absolute alcohol so long as color is given off. The removal of the colored precipitate on the preparation can be hastened by wiping with a fine brush. Alcohol containing water must be avoided. The preparation is then brought into pure xylol and afterwards mounted in balsam or, better, in thickened cedar oil.

Nuclei in general stain blue, part lighter and part darker, part pure blue and part with a tinge of violet. The chromatin threads stain more intensely. The protoplasm of the cell, outside the granulations, show a very diverse staining. The protoplasm of the lymphocytes is mostly bluish, often as dark as the nucleus. Some lymphocytes show a reddish protoplasm. Acidophile granules are generally stained red, neutrophile granules red to blue red, basophile granules are always dark red, many times with a tinge of violet.

ERNEST L. WALKER.

Massachusetts State Board of Health.

## Methods in Plant Physiology.

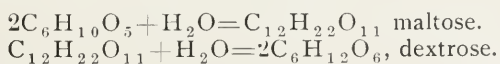
### IX. ENZYMES.

Mention has been made previously of a substance which has the power of converting starch into soluble carbohydrates; this substance, diastase, is one of a large group called enzymes, or unorganized ferments, in distinction from the organized ferments or micro-organisms. Enzymes in general have the power of producing chemical changes in organic compounds without undergoing change themselves. In the study here outlined diastase is used as a typical enzyme.

**1. Preparation of Diastase.** Weigh out 25 g. ground malt and place it in a flask with 100 c. c. of water. (The ground malt may be obtained at any brewery.) Shake the bottle occasionally during the next half hour, then turn the contents out into a mortar and grind for ten minutes. Filter off the liquid into a bottle and add a few drops of chloroform, to prevent the growth of fungi in the solution.

If a purer form of diastase is desired it may be precipitated and dried. Add 95 per cent. alcohol to the aqueous solution of diastase so long as it causes a flocculent precipitate, ceasing when it only renders the solution turbid. Filter out the precipitate, wash it with absolute alcohol, and dry in a dessicator over sulphuric acid. This solid is impure diastase, it may be prepared for use by dissolving in water.

**2. The Hydrolysis of Starch by Diastase.** Under the action of diastase starch is first converted into maltose and dextrin and then into dextrose, grape sugar. The steps in the process are probably as follows:



The operation of hydrolysis is conducted as follows: Prepare some starch paste by adding 1 g. of potato or wheat starch to 100 c. c. of water. Place the flask containing the mixture on a water bath and keep it at the boiling point for

fifteen or twenty minutes. Divide the resulting starch paste into three equal lots. Cool the first lot to a temperature between  $5^{\circ}$  and  $10^{\circ}\text{C}$ . and add 10 c. c. of the diastase solution; set the flask in a place with a temperature of about  $10^{\circ}\text{C}$ . Pour a few cubic centimeters from the second lot into a test tube and add a few drops of iodine solution, note the strong starch reaction. Test another sample with Fehling's solution, for reducing sugar; they will be found absent. Keep the temperature of this flask between  $25^{\circ}$  and  $30^{\circ}$ , add to it 10 c. c. of diastase solution and a few drops of chloroform; test immediately as before for starch and sugar. Observe the progress of the enzymatic action by

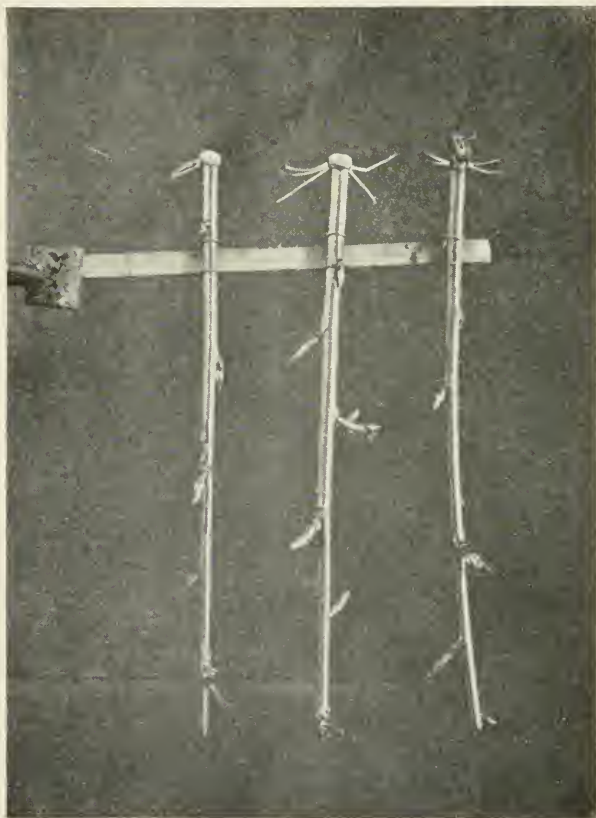


FIG. 9.—Willow cuttings grown in the inverted position. Note the well defined callus on the morphological lower ends.

making similar tests at the end of 5 minutes, 15, 25, 40, 60, 90, and 120 minutes. At less frequent intervals test the progress of enzymatic action in the cooler temperature. Note the total time required in each case for the complete hydrolysis of the starch.

The third flask of starch paste is to be hydrolysed to sugar by boiling with dilute acid. Place the flask on the water bath and add three or four drops of hydrochloric or sulphuric acid. After it has reached the boiling point, at inter-

vals of five to eight minutes take out a few drops of the acid mixture with a glass rod and test for starch with iodine. At intervals of ten minutes test for sugars with Fehling's solution; but before testing neutralize each sample with potassium hydroxid, using litmus paper as an indicator of the neutral condition.

To observe the action of diastase upon normal starch grains, place a small amount of wheat starch and a few cubic centimeters of diastase solution in a well covered dish. Acidify the mixture by the addition of a hydrochloric acid so that it is about .05 per cent. acid. At the end of twenty-four and of forty-eight hours examine microscopically to observe the corrosion of the individual grains.

#### X. CORRELATIONS BETWEEN DIFFERENT PARTS OF PLANTS.

There is often manifested in the growth of new shoots an internal reciprocity which has been termed the correlation of growth. These processes are especially induced by the mutilation of plants. One of the most convenient subjects for this study is Willow or Poplar cuttings; preferably they should be used in the spring before the leaves start. Before placing the cuttings in the culture jars they should be washed quickly in a weak solution of mercuric chlorid and rinsed in distilled water, otherwise they are likely to become infested with fungi.

1. **Polarity of Stems.** (A) Make five cuttings of similar length and fasten them upright in a battery jar; allow the lower ends to dip about 1 cm. into water in the bottom of the jar. The sides of the jar are to be lined with filter paper and the jar is to be covered. Note the regularity with which leaves and roots develop on the upper and lower ends respectively.

(B) Make a preparation precisely similar to the preceding, except that the shoots are to be inverted with their morphologically upper ends dipping into the water. Note the marked polarity of the stems as shown in Fig. 9.

(C) Five similar shoots are to be planted with the morphologically lower half embedded in clean sand in a flower-crock which sets constantly in water. The projecting ends of the shoots should be covered with a sheet of wet filter paper and a bell-jar placed over all. The result is practically the same as (A).

(D) A similar preparation to the foregoing is made, except that the morphologically upper half of each cutting is embedded in the sand. Compare results with (B).

2. **Formation of Roots and Leaves in Response to Injury by Girdling.** The girdling experiments give one an idea of the paths of water and of plastic material in the stem. The results in each case are to be compared with No. 1 (A).

(A) Make a preparation similar to No. 1 (A), cutting out in the center of the stem a complete ring of bark 1 cm. in length. See that all of the bark and phloem are removed, leaving only the wood beneath.

(B) Make another preparation similar to the preceding, except that the bark is removed one-third the distance from the lower end of each cutting.

(C) Make a preparation similar to the foregoing, except that the bark is to be removed one-third the distance from the upper end of each cutting.

The resultant growth in such cuttings is shown in Fig. 10; roots are formed where the downward flow of plastic material is stopped by the removal of the

phloem. So long as the xylem cylinder is left the upward flow of water is not disturbed.

3. **The Influence of Light on the Formation of Roots and Shoots.** (A) Make a preparation similar to No. 1 (A) and exclude all light. The cuttings will show a tendency towards the suppression of shoots and the production of roots.



FIG. 10.—Willow cuttings from which a ring of bark has been removed.

(B) A preparation similar to the foregoing is made, except that light is admitted on one side. Here there is a tendency to produce shoots on the illuminated side and roots on the opposite side of each cutting.

University of Michigan.

HOWARD S. REED.

DR. FREDERICK D. HEALD, professor of biology in Parsons College, Iowa, has been elected to the position of adjunct professor of plant physiology and general bacteriology in the University of Nebraska. He will take up the duties of his new position during the summer, having charge of the classes in botany at the summer session.

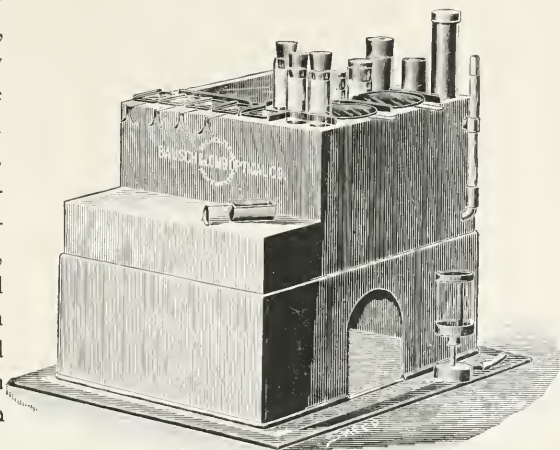


## Elementary Medical Micro-Technique for Physicians and Others Interested in the Microscope.

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### XIV. SIMPLE PATHOLOGICAL TECHNIQUE.—Continued.

*Paraffin Embedding.* This method of embedding offers special advantages in that when the object is embedded it is not necessary to store it in alcohol or wet it or the knife when sectioning it. Furthermore, much thinner sections may be cut. Fix the specimen in Carnoy's fluid and transfer the specimen to absolute alcohol, the same as previously described. Transfer the specimen from absolute alcohol, removing the adherent alcohol with filter paper, to anilin oil until it becomes very clear and sinks. Wipe off the anilin oil and put the specimen in xylol ten to twenty minutes. Transfer to xylol, in which paraffin of  $43^{\circ}$  centigrade has been dissolved to saturation, for an hour, after which it should be placed for two hours in melted paraffin of  $54^{\circ}$  centigrade, which should be kept in a paraffin bath or oven at the lowest temperature at which the paraffin will remain melted. It is now ready to embed. Wrap a piece of letter paper around a homeopathic vial or block of wood that is larger than the specimen so that the paper projects about an inch, making a cup of this depth. Pour full of the melted paraffin, and with a warmed forcep rapidly transfer the specimen to the cup, arranging it with the side to be cut towards the bottom. Blow on the paraffin and when it has filmed over immerse it in cold water to harden, after which the paper may be removed, the block taken from the bottle and clamped in the microtome holder, the excess of paraffin from the deep cell affording a good holding place. Set the knife to cut at right angles to the specimen. Trim the paraffin block square so that one of the straight edges will be presented to the knife. Cut dry. The sections should range from six to twelve microns in thickness. Transfer the sections to xylol to remove the paraffin, then to alcohol to remove the xylol, then to water, after which they may be stained and mounted the same as celloidin sections.



Paraffin or Water Bath.

Staining on the slide is a much better method of handling paraffin sections, as it reduces to the minimum the loss of cells that is likely to occur in the watch-glass method after the supporting paraffin is removed.

Apply to a clean slip a thin film of Mayer's albumin prepared as follows :

White of egg	-	-	-	-	-	50 c. c.
Glycerine	-	-	-	-	-	50 c. c.
Salicylate of soda	-	-	-	-	-	1 gram.

Shake well together and filter.

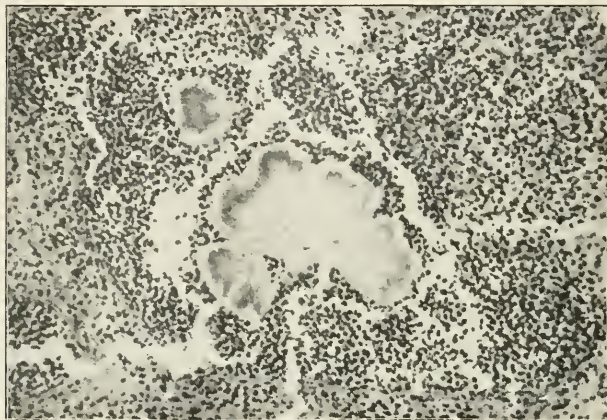


FIG. XXVIII.—*Aetinomyces bovis*. Section. Stain, Ehrlich-Biondi-Heidenhain triple mixture. Magnified 150 diameters;  $\frac{2}{3}$ -inch objective; Bausch & Lomb compensating photo ocular No. 2.

Lay a section on the film, carefully press it down on the film with the finger, which should be dry. Heat the slip carefully till the paraffin melts, then immerse in turpentine, or better xylol, to remove the paraffin, pass the slip from

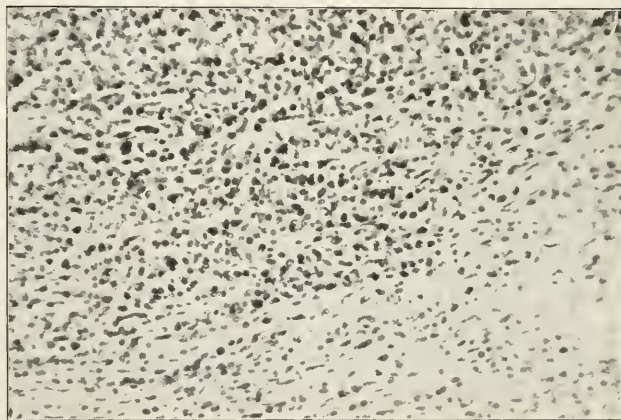


FIG. XXIX.—Round Cell Sarcoma. Stain, borax carmine. Magnified 150 diameters;  $\frac{2}{3}$ -inch objective; Bausch & Lomb compensating photo ocular No. 2.

xylol to absolute alcohol to remove the xylol, then to water. Apply the stain by flooding the slip with it, wash and counterstain in the same way, clear with a mixture of clearing oil, apply balsam and a cover.

The section will adhere to the slip through all these operations.

## Laboratory Outlines for the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

### XXVIII. *Chara* Sp.

Class and order, Charales. Family, Characeæ. Stoneworts.

The stoneworts are algæ which are found growing in the bottom of ponds, lakes, or slowly flowing creeks and rivers. They are of considerable size and are usually covered with an incrustation of lime. They contain numerous branches arranged in whorls and are firmly fixed in the mud by means of rhizoids. Charas grow very readily in an aquarium and may be kept in a healthy condition all winter by simply placing the plants into a glass jar of water and keeping them near a south window.

1. Sketch an entire plant and describe the naked eye characters. Notice the odor, the nodes and internodes, and the brittleness of the filaments.

2. Mount the base of a plant in water and examine under low power. Draw and describe some of the branching rhizoids. Study the rotation of the protoplasm under high power.

3. Mount the terminal part of a young branch, being careful so as not to crush the brittle lateral branches. Examine under low power and draw the terminal bud. Notice the great internodal cells covered with a cortical layer and the whorls of lateral branches.

4. Draw a cell of one of the branches without a cortical layer, showing the incrustation of lime.

5. Under high power draw part of a cell, showing the chloroplasts. How are they arranged? Draw several in stages of division. How do they divide?

6. Study the rotation of the cytoplasm in the large cells of the branches and describe. How does it differ from that in the cells of *Phylotria*? Why are the chloroplasts arranged in rows? Note the movements in opposite directions on either side of the neutral line. Is the direction of rotation the same in all the cells?

7. How is the cortical layer developed? In order to determine this, young branches should be observed. Draw a cross section of the main stem. Note the short projecting cells which roughen the surface.

8. The sexual organs are produced during summer and autumn. Study fresh material, or if this is not at hand, material preserved in alcohol or Petit's solution. The antheridia and oogonia are situated on the lateral branches. Draw. Notice the five spiral branches which cover the oogonium. How does this oogonium differ from that of *Vaucheria*? The antheridia are globular organs which are red in color when fresh. Is this plant hermaphrodite or unisexual?

9. Draw an oogonium containing a ripe oospore. Explain the structure of the entire body.

10. Crush a ripe antheridium under the cover-glass and draw one of the numerous filaments inside. The small cells of these filaments contain the spermatozooids. Draw a single cell showing a mature spermatozoid. How many cells in a single filament? Suppose the antheridium contains  $8 \times 6 \times 4$  filaments, how many spermatozooids would there be produced in each antheridium? How many spermatozooids for each oospere or egg?

11. Study the proembryo, from which the normal Chara plant develops as a lateral bud. Draw and describe. Proembryos may be obtained by placing plants with mature spores in a glass jar of water and keeping them over winter. In the spring the embryos will be found at the bottom.

12. Make a diagram showing the life cycle of Chara. Compare with Vaucheria.

XXIX. *Batrachospermum moniliforme* Roth.

Class, Florideæ. Order, Nemalionales. Family, Helminthocladiaceæ.

Batrachospermum is an alga of considerable size which can be found attached to stones in fresh water rivulets and creeks.

1. Spread out the frond of the alga in water in a porcelain plate and sketch the entire plant.

2. Mount some of the branches in water, crushing them considerably under the cover-glass, and sketch under low power.

3. Under high power draw one of the lateral branches coming out from the nodes. Note the oval cells and the bristle-like projections on some of the terminal cells.

4. In a young, main branch study the branches which pass down from the base of the nodal branches and form a loose cortical layer. How does this compare with the cortical layer in Chara?

5. Crush some of the older branches under the cover-glass by pressing and rubbing carefully over the surface with the handle of the needle and study the oögonia. These are situated on the lateral branches, and each consists of a thickened hair-like process (trichogyne) and a bulbous base (trichophore) containing the oöspere. Draw.

6. Study the antheridia, which are single terminal cells, each of which develops a single spherical male gamete (spermatium) without flagella. Draw an antheridium and a free floating spermatium.

7. Draw a oögonium which has one or more spermatia attached to the trichogyne.

8. Draw a sporocarp under low power. This is a spherical cluster of branches which develops from the fertilized egg.

9. Under high power draw a nonsexual carpospore at the end of one of the branches of the sporocarp.

10. From the foregoing study it will appear that Batrachospermum possesses a sort of an alternation of generations. Besides this, it has another interesting stage. When the carpospore germinates it gives rise to a peculiar filamentous proembryo or protonema, formerly known as the chantransia stage, from which the normal Batrachospermum plant develops as a lateral bud. This is a case of



polymorphism which is probably dependent on physical conditions. Protonemal plants should be collected showing various stages in the development of the *Batrachospermum* bud. The chantransia filament can reproduce itself by means of nonsexual spores developed on the tips of its branches. This is a case of reproduction known as paedogenesis, since the process is accomplished while the plant is in the immature condition. If material is at hand, draw and describe the chantransia filaments and spores.

XXX. *Polysiphonia variegata* (C. Ag.) J. Ag.

Class, Florideæ. Order, Rhodymeniales. Family, Rhodomelaceæ.

*Polysiphonia* grows in abundance on rocky sea coasts. The plants may be found in summer as purplish-brown tufts, a few inches long, on other larger water-plants, or on piles and stones. Preserved material may be used by those living away from the seashore.

1. Spread out a frond in a porcelain plate and sketch the entire thallus. Note the hold-fast, if present, and the mode of branching.

2. Mount a branch and draw under low power. Note that the body of the thallus consists of successive tiers of cells, each tier consisting of a central cell, surrounded by a layer of cortical cells.

3. Under high power draw a single tier of cells. Crush the thallus a little and note especially the large protoplasmic strands (protoplasmic continuity) which run from the central cell to the several cortical cells of the tier. Note, also, the strands connecting the cells of a tier with those of the tiers above and below.

4. Cut cross sections of the thallus, mount, and study under high power. The sections may easily be obtained by chopping up a moist branch on a piece of paper with a sharp scalpel. Draw, showing the arrangement of the central and cortical cells and also the protoplasmic connections.

5. Under high power study the tip of a young branch and draw. Notice the dome-shaped apical cell and a number of cells below. The lower ones are divided by longitudinal walls. How are the tiers and the cortical cells developed? From this it is evident that, although *Polysiphonia* appears like a branched filament and continues to develop as such, it finally forms a true solid aggregate.

6. Nonsexual spore reproduction. Mount branches containing tetrasporangia (dark spherical bodies below the cortical cells) and draw under high power. Draw one of the mature spores.

7. Sexual reproduction. The antheridia are borne on delicate, colorless dichotomously branched filaments, which form tufts on the younger part of the frond; the oögonia are on short branches in the upper part. Mount branches containing antheridia and draw under high power. Note the slender tip of the branch which extends beyond the oblong antheridium.

8. Development of the oögonium. Mount branches containing young oögonia and under high power draw: (*a*) a short lateral simple branch showing one of the cells considerably enlarged and more or less spherical; (*b*) one in which this cell has divided by vertical cells into four cells; (*c*) one in which the inner cell of the tier of four has enlarged and divided into three or four cells by transverse walls, the upper one developing into the oögonium with a basal trichophore and a slender trichogyne; (*d*) a young cystocarp showing the trichogyne protruding from the mass of cells forming the wall.

9. Draw a mature cystocarp, showing the more or less ovate-globose wall and the carpostome.

10. Crush one of the mature cystocarps and draw several of the dark colored, nonsexual carpospores. *Polysiphonia* has an alternation of generations, since the spore-bearing part of the cystocarp is homologous to the sporophyte of higher plants.

JOHN H. SCHAFFNER.

## LABORATORY PHOTOGRAPHY.

L. B. ELLIOTT.

Devoted to Methods and Apparatus for Converting an Object into an Illustration.

### A HINT FOR OVER-EXPOSED SLIDES.

Many people will find that some of their slides are over-exposed. This is seen by the image coming up and at once rapidly darkening. If the slide was now fixed it would be flat and of bad color. Instead, however, of removing the slide from the developer and fixing, continue developing it until it is very dense and very much over-developed. Then, after fixing, the slide must be placed wet with hypo. into a bath of ferricyanide of potassium. To prepare this bath add a few crystals of the above salt to a dish of water—in fact, enough to make the solution a canary yellow. The slide in this bath rapidly reduces, and the operation must be continued till the density is considerably thinner than a normal slide should be. Wash thoroughly to get rid of hypo., and bleach the slide thoroughly in the following—in fact, it will be impossible to over-bleach it: Mercuric chloride, 50 gr.; potass. bromide, 50 gr.; water, 5 oz. After this, wash well to get rid of the mercuric chloride and blacken with ammonia (.880), 1 oz., water, 20 oz. The slide will now be found to be crisp, with perfect gradation and a very pleasing color.

This method is particularly useful for getting good slides from very thin negatives, only care must be taken in the first place not to over-expose. For sea-scapes, if the exposure is made so as to get a black tone, and the slide is under-developed, and after fixing and washing well is only intensified, a most intense beautiful purple black will be produced, almost as fine as wet collodion.  
—*Photographic News*.

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ON USING BROMIDE.—A bottle of 10 per cent. bromide of potassium should find a place in every careful worker's dark-room, in case of sudden fog appearing, or when it is manifest that the exposure has been excessive. A drop or two added to the developer under such circumstances will often save a plate which, under normal circumstances, would find its way to the dustbin. Bromide should not be kept in an ordinary bottle, but should be stored in a "drop bottle," or similar device. A very good substitute, and one which will answer quite as well, if not better, is found in the form of a small bottle having a hole bored in the cork of sufficient size so that a fountain-pen filler can be fitted tightly into it. The filler is allowed to dip to the bottom of the bottle, and any time that a drop or so of bromide is required, it is a very easy matter to compress the india rubber, withdraw the cork and tube from the bottle, and discharge the necessary number of drops.—*Ex.*

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Shibata, K. Experimentelle Studien über die Entwicklung des Endosperms bei *Monotropa*. (Vorläufige Mittheilung) Biologisches Centralblatt 22: 705-714, 1902.

Prof. Shibata records important observations upon the embryo-sac structures of *Monotropa uniflora* L. Most of

the work was done with living material. The plants were kept in glass dishes with a little damp Sphagnum, and were kept covered with glass bell-jars. To prevent fertilization the stigma was cut off and the cut surface covered with paraffin. From time to time small pieces of the ovary with its ovules were removed, and here also the cut surfaces were covered with paraffin. When a sterilized knife is used and the cut surfaces are thus covered with paraffin, no damage is done by the operations, and a series of stages obtained from the same ovary may be studied in the living condition.

Some of the results already obtained are as follows: The interval between pollination and fertilization is dependent upon temperature. Under normal conditions fertilization takes place about five days after pollination, and on the same day, or the next day, from two to four cells are found in the endosperm. After fertilization the egg elongates and breaks into the nearest endosperm cell. Seed ripens in fifteen days after pollination. Light, atmospheric pressure, and mechanical injury of the ovule or surrounding structures exert no influence upon fertilization and subsequent phenomena. At 28° C. fertilization and subsequent phenomena take place as at room temperature, and even at 30° C. the endosperm nucleus can still divide, but at 31-32° C. fertilization can no longer take place and various disturbances are seen in the embryo-sac. By lowering the temperature the interval between pollination and fertilization is lengthened, and at 8-10° C. fertilization is prevented.

The experiments show that the polar nuclei fuse even in the absence of pollination, but the fusion is hastened by pollination even if fertilization be subsequently prevented by removing the portion of the style which contains the pollen tubes. Normally, the polar nuclei fuse about five days after pollination, but when pollination is prevented, the interval may be ten days or even three weeks.

The three small antipodal cells disintegrate after fertilization, but when fertilization is artificially prevented they enlarge and become the most conspicuous objects in the embryo-sac. Their growth is entirely checked at a temperature of 30° C.

Development of the endosperm can be induced experimentally in the absence of fertilization, although in this case most of the ovules collapse. In the rest, about 3-5 per cent., the development of endosperm begins about two weeks later than when fertilization is allowed to occur.

C. J. C.

**Strasburger, Ed.** *Das botanische Practicum*.  
Fourth edition; 8vo, pp., L + 771, 230 figs.  
Gustav Fischer, Jena, 1892. Price, 20 marks.

This book will be welcomed by all botanists who are able to read German. It seems safe to say that no

botanical text-book of modern times is more fully abreast with the present state of knowledge. While the new edition preserves the general arrangement and sequence of subjects which have proved satisfactory in the previous editions, each subject has been carefully revised and brought up to date, even the latest investigations upon protoplasmic connections receiving attention. The latest methods in technique are also presented.

The indices are more extensive than in any of the previous editions, occupying 145 pages. The following are the five indices:

- I. An alphabetical index of the plants which are used as illustrative material.
- II. A list of plants used as illustrative material arranged with reference to the time of year at which they should be collected.
- III. A list of stains and reagents.
- IV. Reagents, stains, plant substances, embedding media, sealing media; chemical exercises; instruments, apparatus and their use; making of preparations; physical exercises.
- V. General index.

The first two indices will be suggestive and helpful to those who have the responsibility of keeping laboratories supplied with illustrative material. The fourth index is so full that in very many cases it will hardly be necessary, especially for the well informed teacher, to look up the reference to the body of the book.

This book is not to be confused with the *Handbook of Practical Botany* by Dr. E. Strasburger, which is the fifth English edition of a translation by Hillhouse of an earlier edition of *Das Kleine botanische Practicum*. Some of the defects of this English edition, which bears Prof. Strasburger's name, although in many cases it does not represent his views, have been noted in the May (1902) number of the JOURNAL.

C. J. C.

**Murbeck, Sv.** Ueber die Embryologie von  
*Ruppia rostellata* Koch. Kongl. Svenska.  
Vetenskaps-Akademiens Handlingar, 36:  
1-21, pls. 1-3, 1902.

Material of this decidedly hydrophytic plant was fixed in Flemming's solution and also in Keiser's corrosive subli-

mate-acetic acid solution. The former, followed by safranin and gentian violet, proved to be better for stages up to fertilization, while Keiser's formula, followed by fuchsin and iodine green, gave better preparations for the study of the embryo.

During the development of the microspores, the tapetal cells break down and their nuclei float free in the cavity of the microsporangium. The formation of two definite male cells within the irregularly elongated pollen grain was traced in detail. A tapetal cell is formed and the megaspore mother cell gives rise to four megaspores, not arranged in a row, but the two lower spores lying one above the other, and the two upper ones lying side by side or somewhat obliquely. The arrangement was observed in numerous instances. In one ex-



ceptional case the archesporial cell seems to have divided obliquely, instead of by the usual pericline, and both the resulting cells show the distinguishing characters of megaspore mother cells. In the mitosis by which the archesporial cell gives rise to the tapetal cell and the megaspore mother cell, the number of chromosomes was found to be sixteen. This number was counted in other sporophytic cells. At the first division of the megaspore mother cell, and at the corresponding division of the microspore mother cell, the number of chromosomes is eight.

The polar nuclei fuse completely before fertilization. Although the pollen tubes were carefully traced to the embryo-sac, the actual process of fertilization was not observed. At the first division of the endosperm nucleus a wall is formed, separating the sac into two chambers. The nucleus in the chamber at the antipodal end does not divide, but the nucleus in the other chamber divides repeatedly, giving rise to numerous free nuclei which are never separated by walls.

A study of the embryo confirms the account of Wille that a primary root is formed at the base of the embryo, but is soon disorganized, and a lateral root which is formed very early is the first functional one. This is very different from the account of Ascherson in Engler and Prantl's *Die natürlichen Pflanzenfamilien* and followed in Goebel's recent *Organography*, according to which this lateral root is the primary root, its unusual position being due to displacement.

C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

**Hunter, S. J.** Artificial parthenogenesis in *Arbacia* induced by the Use of Sea-water Concentrated by Evaporation. *Am. Jour. Physiol.* 6: 177-180, 1901.

The use of this solution he found to produce artificial parthenogenesis in the sea urchin *Arbacia*. Sea-water of perceptibly greater or less osmotic pressure than this will not produce artificial parthenogenesis. Indications point to the need of a certain definite osmotic index or degree of pressure for the result. These observations confirm Dr. Loeb's work in an interesting manner.

**Loewenthal, N.** New Alcoholic Carmin Solution. *Zeit. wiss. Mikr.* 19: 56-60, 1902. Review in *Jour. Royal Micr. Soc.*, Dec., 1902, pp. 715-716.

The author condensed sea-water until it was isotonic with Loeb's 10-15 per cent.  $2\frac{1}{2}$  n. sodium chloride. The

A sodium picro-carmin mixture is made by heating together 0.4 grm. carmin, 100 c.c. water, and 8 c.c. of 10 per cent. caustic soda. While still hot, 25 c.c. of

.5 per cent. aqueous solution of picric acid are gradually added. When cold this sodium picro-carmin is mixed with half its bulk of 1 per cent. HCl. The red precipitate which forms is washed till the water is no longer yellow. The dark red deposit on the filter is then dissolved in 70 per cent. alcohol acidulated

with HCl (about 1 per cent.). The solution is quite clear, and gives good results after any method of fixation. The immersion time is from one-half to several hours. The after treatment is simple, and consists of changes of alcohol from 70 per cent. up to absolute. The author states that the solution is an effective nuclear stain, that by its use aqueous media can be avoided, and that it does not color celloidin.

**Boveri, Th.** Das Problem der Befruchtung. Jena, 48 pp., 8vo, 1902. Review in Jour. Royal Micr. Soc., Part 4, 418-419, 1902.

Boveri in this lecture tersely reviews the status of the processes of fertilization, with a critical discussion of the

meaning of the various steps. The most important part is, perhaps, his modification of the view that the chief function of the spermatozoon is to import a centrosome into the inert ovum. Morgan and others showed that alterations in the saline composition of the sea-water resulted in the appearance of bodies like centrospheres in the ova of sea urchin, etc. Loeb showed that in similar conditions artificial parthenogenesis resulted. Wilson noticed that in Loeb's experiments bodies like centrospheres appeared in the unfertilized ova, and seemed to initiate the segmentation; Boveri now suggests "that it is not a centrosome as an organized structure that is introduced into the egg, and there starts the segmentation process, but rather a chemical substance, which in combination with the ovian cytoplasm, produces the body in question." Such a view would reconcile much that has hitherto been difficult of explanation in connection with the diverse behavior of centrosomes in different organisms, and even in different cells and tissues of the same individual.

**Michaelis, L., u. Wolff, A.** Ueber Granula in Lymphocyten. Virchow's Arch., 167: 151-160, 1 tf., 1902.

The stains used hitherto only differentiate the cell-body of the lymphocyte.

The lymphocyte protoplasm is basophilic. Pappenheim's methyl-green-pyronin mixture differentiates the nucleus and protoplasm. Romanowski's methylen-blue-eosin goes further. For this method two stains are necessary: (1) A methylen-blue solution containing 1 per cent. methylen-azure, made as follows: 200 c.c. of a 1 per cent. solution of methylen-blue is boiled with 10 c.c. of  $\frac{1}{10}$  per cent. sodium hydroxide for a quarter of an hour, and when cool is neutralized with 10 c.c. of  $\frac{1}{10}$  per cent. test sulphuric acid. (This solution is to be obtained from Grübler, in Leipzig, and Leitz, in Berlin, under the name of "azure-blue.") (2) An aqueous eosin solution 1:1000. Immediately before use mix 2 c.c. of the azure-blue solution with 10 c.c. eosin solution, pouring the mixture back and forth several times to ensure complete stirring. It is used for staining regardless of the precipitate formed. To avoid getting this precipitate on the cover-glass the mount is stained in a glass with a concave bottom and placed, face downward, for fifteen minutes, then the preparation is washed off in a very strong stream of water and dried. Those mounts were most satisfactory which had been fixed for an hour in absolute alcohol. Reuter has lately used the precipitate arising from the methylen-blue-eosin mixture as a stain. Each expanded lymphocyte shows, with this stain, a delicate sky-blue protoplasmic cell-body surrounding the violet-red nucleus. In this cytoplasm are seen violet granules as yet undemonstrated by any other method.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

Looss, A. The Sclerostomidæ of Horses and Donkeys in Egypt. Records Egypt. Gov. School of Medicine. 1: 25-141, 13 pls. 1901.

The nematodes are found in the cæcum and large loop of the colon. In collection the practice was to ligature the

gut at intervals, slit it longitudinally, and roll out the contents without disturbing the original position of the parasites near or attached to the intestinal wall. The worms should be preserved immediately upon collection, for they contract and wrinkle upon cooling. The Sclerostomidæ, and the greater part of other nematodes, are easily fixed in an extended state by placing them in 70 per cent. alcohol, heated to 80°C. Contracted individuals will extend when placed in the hot fluid. A few forms, such as *Trichocephalus*, show a tendency to coil up when placed in the alcohol. This may be prevented by grasping the worm between the blades—not the points—of a pair of forceps and shaking out the coil in the killing fluid. This method of killing avoids shrinkage, and gives fine histological details. The worms may be kept permanently in alcohol of this grade. Formalin preserves these nematodes well, renders them somewhat transparent, but interferes with subsequent treatment by alcohol, which causes unavoidable shrinkage.

For the microscopical study of the worms killed in alcohol it is necessary to clear the tissues. This is accomplished by adding 5 to 20 per cent. of glycerine to the 70 per cent. alcohol in which the worms are placed, and allowing the alcohol to evaporate slowly. The weaker solutions of glycerine and slow evaporation must be used with the more resistant species. The water should also be evaporated by placing the preparations in a water bath. Small and less resistant species may be placed directly in 20 per cent. glycerine solution, and in a day are ready for evaporation in the water bath. Material killed in various standard reagents resists the glycerine solutions, and at the best is less transparent than that fixed in hot alcohol. Worms thus cleared in glycerine exhibit their internal structure very well. The alcohol-glycerine mixtures may be heated and used in place of the alcohol for fixing, thereby reducing the time in preparation. Small worms killed in the 20 per cent. glycerine and placed at once in the water bath are ready for examination in 24 hours.

Worms from the pure glycerine may be brought directly into 96 per cent. alcohol without shrinkage, and carried through cedar oil to paraffin. The cuticle should be slit in several places to facilitate the passage from absolute alcohol to oil. Cedar oil is very highly recommended by the author for clearing preparatory to paraffin. It penetrates quickly without shrinkage (in incised specimens) and does not cause brittleness. Sections and whole preparations were mounted in glycerine gelatin to avoid the transparency caused by balsam.

C. A. K.

**Meisenheimer, J.** Beiträge zur Entwicklungs-  
geschichte per Pantopoden. 1. Die Ent-  
wicklung von *Ammotha echinata* Hodge bis  
zur Ausbildung der Larvenform. Zeitsch.  
f. wiss. Zool. **72**: 191-248; Taf. 13-18, 1902.

Eggs were fixed in cold Hermann's and  
in Zenker's fluids, and both gave good  
fixation. The former, however, black-  
ened the yolk granules so intensely

that the nuclei and cell boundaries were obscured. The use of iron hæmatoxylin was avoided for the reason that it also stained the yolk so deeply as to obscure other structures. After many experiments with stains, Mayer's hæmalum was found to be the most satisfactory stain, since it left the yolk almost colorless, while nuclei and cell boundaries were sharply marked out. Alum carmine was used for *in toto* preparations. Hoffmann's method of orienting these small objects was employed to secure sections in definite planes. C. A. K.

**Strong, R. M.** The Development of the De-  
finitive Feather. Bull. Mus. Comp. Zool.  
**40**: 147-184, 9 pls., 1902.

Remiges of *Sterna hirundo* plucked  
from the young bird were fixed in  
picro-sulphuric mixture or Hermann's

fluid, the latter giving better fixation. For infiltration of this material, and of dry feathers with paraffin, the chloroform method was used. Other clearing agents caused the sections to fall out of the ribbon. Feather germs require 2-5 days' infiltration in melted paraffin. Twelve hours suffices for dry feathers. Hard paraffin (135°F.) or paraffin to which 5 per cent. resin was added, was employed, and with this it was possible to secure 2  $\mu$  sections. Cornified portions of the sections, especially of those as much as ten micra in thickness, tend to curl and spring from the ribbon, and with osmic acid material it is necessary to use, in addition to the albumen fixative, a film of collodion, applied immediately after the xylol is removed. Kleinenberg's 70 per cent. alcohol hæmatoxylin was used for staining, and followed by eosin, which reddens cornifying tissues. Teased preparations of Hermann's material similarly stained *in toto* were found to be very instructive. C. A. K.

**Ikeda, I.** Observations on the Development,  
Structure, and Metamorphosis of Actino-  
trocha. Journ. Coll. Sci. Imp. Univ.,  
Tokyo, Japan, **13**: 507-592, pls. 25-30, 1901.

Colonies of *Phoronis ijimai* were found  
under overhanging ledges of rocks  
accessible at low tide. Mature sexual

elements were found from November to June, and during the greater part of the year eggs and young embryos were found clustered in "embryonal masses," adhering to the lophophoral crown of the adult on either side of the median line. Stages from fertilization to the early larva were secured here. Older larvæ were taken in the surface tow-net. Saturated solution of corrosive sublimate in 1 per cent. acetic acid or Flemming's fluid was used for fixing material. Delafield's hæmatoxylin, with eosin or safranin for counter-stain, was used for staining sections. C. A. K.

**Beguin, Felix.** Contribution à l'étude histolo-  
gique du tube digestif des Reptiles. Rev.  
Suisse Zool. **10**: 251-399, pls. 4-9, 1902.

The mucus membrane of the intestine  
changes very quickly after death, so  
that tissues for histological prepara-

tions must be fixed immediately, or while still living if possible. The author used sublimate + 10 per cent. glacial acetic acid for 30 minutes or less, or picro-nitric for 2 hours, or Zenker's fluid. For maceration in 30 per cent. alcohol tissues were prepared in 1 per cent. osmic acid. For staining, Mayer's acetic hæmalum was used with counterstain of eosin, safranin, or Bismarck brown. Borax carmine was also counterstained with Bismarck brown. The eosin serves to define cell contours, and the safranin or Bismarck brown color the least trace of mucus in the cells. C. A. K.



## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoological Laboratory,  
University of Michigan, Ann Arbor, Mich.

Tangl, F. Beiträge zur Energetik der Ontogenese. I. Mitth. Die Entwicklungsarbeit im Vogelei. Arch. f. d. ges. Physiol. 93: 327-376, 1903.

The problem of the energy relations involved in the various physiological processes going on in the organism is one which has been given but com-

paratively little general attention by animal physiologists. Tangl has undertaken the investigation of one phase of the problem, viz., the determination of the amount of energy involved in the processes of ontogenetic development, and in this paper are contained his first results from the study of birds' eggs. He calls the total amount of chemical energy which is transformed during the development of the embryo the "Entwicklungsarbeit" (work done in development), and proceeds to find by careful measurements what the amount of this energy in a given case is. The principle of the method of determining the "Entwicklungsarbeit" is as follows: The energy content of the egg is determined at the beginning and at the end of development. Then the difference in these two quantities indicates the amount of energy which has been transformed and utilized in the development. As material the eggs of the sparrow and of several varieties of hens were used. The experimental procedure in the case of the sparrow eggs was as follows: An egg was first weighed and measured: then broken into a clean watch glass of known weight, and the embryo measured. The eggs and shell were then dried in a vacuum at 50°—60° C. Thus the weight of the dry substance was obtained, and from this dry substance pastilles were made in a pastille press. These pastilles were then burned in a Berthelot Mahler calorimetric bomb, and the energy content of the dry substance obtained. The procedure when hen's eggs were used differed from this only in minor details. It was found that in the complete development of the sparrow embryo the work done amounted to 755 gram calories, or in mechanical units  $3.16 \times 10^{10}$  ergs. For the development of the hen's egg up to the time of hatching 16 kilogram calories were used, or in mechanical units  $66.9 \times 10^{10}$  ergs. In the complete development of each gram of the embryo 658 gram calories are transformed, and for each gram of dry substance of the developed embryo 3426 calories of chemical energy have been transformed. It appeared very clearly in the experiments that a considerably larger amount of energy was used in the early stages of development than in the later stages. There is evidence in the results that indicates that the energy necessary for development comes from the transformation of the chemical energy of the fat in the egg. Measurements of the energy content of different organ systems in the developed embryo showed that the muscles had the highest specific energy content (6687 calories) and the bones the lowest (4907 calories).

**Babák, H.** Ueber die Entwicklung der locomotorischen Coordinations-thätigkeit in Rückenmarke des Frosches. Arch. f. d. ges. Physiol. **93**: 134-162, 1902.

The author opens in the work here reported a new and promising field in the study of the ontogenetic developments of, and ontogenetic changes in

the physiological functions of the different parts of the body. He points out the fact that there has been no thorough systematic investigation of the ontogenetic development of the functions of the nervous system. The present work deals with the development of the function of the locomotor coördination in the spinal cord of the frog. Experiments were performed on the larvæ of different ages and on very young and partially metamorphosed frogs. It was found that in young larvæ and frogs distal (posterior) parts of the spinal cord have very much greater coördinating powers than is the case in the adult, fully grown frog. As development proceeds the locomotor coördinating powers become confined to more and more proximal parts of the nervous system. A parallel relation of course obtains in the phylogenetic series.

R. P.

**Abel, J. J.** On the Elementary Composition of Adrenalin. Proc. Amer. Physiol. Soc. Fifteenth Ann. Meeting. Amer. Jour. Physiol. **8**: xxix-xxx, 1903

Attempts to purify and obtain constant results in the analysis of the very interesting substance adrenalin have not so far been successful. Abel found in a series of analyses the following ex-

**Amberg, S.** The Toxicity of Epinephrin (Adrenalin). Ibid. Pp. xxxiii-xxxiv, 1903.

trems of variation in the content of C, H and N. C = 56.53 to 58.89; H = 4.77 to 7.19; N = 7.59 to 10.65 (Dumas). It was found that frequent reprecipitations of adrenal if dissolved in dilute HCl by means of ammonia or sodium carbonate raised the carbon content of the compound. In the earlier part of the work the analyses gave results which made it possible to deduce for adrenalin the formula,  $C_{10}H_{13}NO_3, \frac{1}{2}H_2O$ , but it was found that the method used in the determination of the nitrogen (Kjeldahl-Gunning) did not give the whole of this element, so that this formula will not hold.

Amberg found that 2.0 mgm. per kg. of adrenalin injected intravenously into a dog caused death. 6.0 mgm. per kg. injected subcutaneously was fatal. The pathological changes produced by the drug consist in hemorrhages, which were observed in the heart, lungs, intestines, peritoneum, in and around the pancreas, liver, adrenal gland and thymus glands.

R. P.

**Gorham, F. P. and Tower, R. W.** Does Potassium Cyanide prolong the Life of the Unfertilized Egg of the Sea-Urchin? Amer. Jour. Physiol. **8**: 175-182, 1902.

About a year ago Loeb and Lewis (Amer. Jour. Physiol. **6**: 305) announced the discovery that if unfertilized eggs of the sea-urchin were kept

for a time in a weak solution of potassium cyanide they retained the power of being fertilized and developing (i. e., lived) considerably longer than eggs left in ordinary sea water. From this they concluded that KCN "prolonged" the life of the egg, by "checking specific mortal processes."

Gorham and Tower find as the result of an extensive series of careful experiments on the eggs of the winter flounder, *Pseudopleuronectes americana* and of the sea-urchin *Arbacia punctulata*, that the action of potassium cyanide in "prolonging" the life of the egg is only an indirect one. The poison kills or inhibits the

bacteria and so provides the eggs with a more favorable environment. By keeping sea-urchin eggs in sterilized sea water they were able to produce plutei from eggs which had been kept eleven days before fertilization. The longest time that Loeb and Lewis were able to keep them before fertilization, using the most favorable KCN solution, was four days. Eggs survived longer in cultures inoculated with protozoa (presumably ciliate infusoria) than in control cultures without protozoa. The result here was again evidently due to destruction of bacteria. The authors' final conclusions may be quoted: "Both our own experiments and those of Loeb show that too strong solutions of potassium cyanide, and too long exposure to weak solutions, soon kill the egg. From this the reasonable interpretation is, that the potassium cyanide is a poison to all living matter, but it acts more quickly on bacteria than on sea-urchin eggs; it is in no sense a prolonger of life. From the fact that unfertilized eggs can be kept in sterile sea water for eleven days or longer, it would seem that the specific mortal processes of Loeb are as yet hypothetical phenomena without any definite experimental basis."

R. P.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn, Wesleyan University, Middletown, Conn.

Report on Diphtheria Bacilli in Well Persons,  
by a Committee of Massachusetts Association  
of Boards of Health.

This committee, composed of a considerable number of bacteriologists of high reputation, have presented, recently,

a report upon the extremely significant topic suggested in the title. The fact that diphtheria bacilli may be found in the throats of perfectly healthy persons is clearly a matter of the utmost importance in relation to the problem of isolation of patients, and the report of this committee is of the greatest value in its relation to the methods of procedure adopted by boards of health. The conclusions reached are briefly as follows: Diphtheria bacilli are frequently found in the throats of perfectly healthy persons, the percentage varying according to the different observers, largely because different bacteriologists use different criteria for determining this bacillus. The percentage given ranged all the way from 1.2 per cent. to 22 per cent. or even more. These instances are found among well people, scattered in general among the public, but are naturally more abundant among members of families in which there are cases of diphtheria. Of the former, it is estimated, according to the percentage, that there must be at least 8,000 persons in the city of Boston harboring bacilli in their throats. The conclusion reached by the committee is that it is quite impracticable to isolate such persons, if they are not known to have been recently exposed to the disease. The presence of persons with bacilli in their throats in the families that have cases of diphtheria is naturally considerably higher. The committee, however, after careful consideration of the matter, conclude also that it is not

advisable, as a matter of routine, to isolate all well persons in infected families, schools, or institutions. One member of the committee differs from this to a slight extent. The importance of these conclusions in determining the rules for quarantine, adopted by health boards, is very manifest. The rest of the paper, which is a rather lengthy one, is devoted to the bacteriological study of the types of bacteria which come under the general group of diphtheria organisms. The authors describe seven types of bacilli, which, in general, may be found grouped together, of which the first three only are regarded as typical, virulent diphtheria bacilli. The report is reprinted from the Journal of the Massachusetts Association of Boards of Health, July, 1902, and is one that should be thoroughly studied by all interested in the problems of public health. H. W. C.

**Van Houten.** A Successful Attempt to Cultivate the *Bacillus Lepræ*. Jour. of Path. and Bac. 8: 260, 1902.

Although many attempts to cultivate the leprosy bacillus have given apparently positive results, the author of this paper is inclined to doubt whether any of them are sure enough to leave it certain that the bacillus has actually been cultivated. He adopts a somewhat new method of study and is confident that he has succeeded in obtaining pure cultures of the bacillus. His method is to inoculate broth with leprosy material, and for this purpose he finds that the best success is obtained by a mixture of fish broth and beef broth, which must be slightly alkaline. In such a broth the bacilli grow readily, many of them showing motility. From such a broth he is able to obtain sub-cultures in a similar mixture, and, after several cultivations, sub-cultures can be obtained in beef broth. The pure cultures agree in morphological characters with the leprosy bacillus, but the chief evidence that he has succeeded in his work is obtained by the action of the leprosy serum upon cultures of the organism. These bacilli, when mixed with diluted human serum, show the Pfeiffer-Bordet reaction. This reaction, which destroys the bacilli, occurs both with ordinary serum and with leprosy serum, but the reaction is much more marked with leprosy serum than with the ordinary human serum, from which he concludes that his organism is the specific agent of leprosy. H. W. C.

**Kasperek.** Cent. f. Bac. u. Par. O. 13: 383, 1902.

Dr. Kasperek has described a very ingenious funnel devised for the purpose of filtering gelatine or agar that needs to be kept warm during filtering. The piece of apparatus consists of an ordinary glass funnel, in which are placed several layers of asbestos paper, fastened together with water glass, the asbestos paper conforming to the shape of the filter. Between the layers of the asbestos there are wound about three meters of a .3 mm. nickel wire, so wound around that the different coils are thoroughly isolated from each other. The two ends of the wire are connected with binding screws. To one of the binding screws is attached an electric wire from an electric current, and to the other a wire that is arranged to pass through a series of ordinary incandescent lights. By connecting the wires with the electric current and turning on a single light, the filter is warmed to a temperature of 42°. By connecting with two lights of similar power the filter is warmed to 60°, and by the addition of a third light of 16 candle power, a temperature of 70° can be obtained. This filter is used in the ordinary way and is extremely convenient for filtering material that needs to be kept warm.

H. W. C.



**Jochmann.** Ueber neuere Nährboden zur Züchtung des Tuberculoseeerregers, etc. Hyg. Rund., p. 969, 1900.

The most vigorous development is obtained from the blood serum of sheep, cattle, and man, to which lactic acid had been added in the proportion of ten drops of a 1 per cent. solution to 50 c. c. of the serum. The above serum should give a neutral reaction with the litmus before the lactic acid is added.

H. W. C.

**Bang.** Ueber die Abtödtung der Tuberkelbacillen bei Wärme. Zeit. f. Thiermed. 7: 81, 1902.

bang has contributed a series of careful experiments to the question as to the temperature at which the tubercle bacilli in milk are rendered innocuous. The claim made by Smith, some years ago, that tuberculous milk, heated for a time to 60° C., in such a way to avoid the formation of the scum, is made incapable of producing tuberculosis, has been strongly contested. The very high rank of Prof. Bang makes it especially interesting to find that he confirms Smith's observation practically *in toto*. He finds that milk in closed tubes, heated to 60° for five minutes, if inoculated, produces tuberculosis, but to a moderate extent; if heated for fifteen minutes, the effect is very much reduced. Milk heated to 65° for five minutes is rendered entirely innocuous, and the same result follows heating at 75°, 80° and 85°. He found, however, that milk heated for two minutes at 60° gave only negative results when this milk was used in feeding experiments, thus confirming the conclusion reached by Smith, that a temperature of 60° for a comparatively few minutes is quite sufficient to render milk incapable of producing tuberculosis when it is subsequently used as food.

A series of experiments on the same subject has been described by Hesse in Zeit. f. Thiermed., p. 321, 1902, and the results were practically identical, showing that a heating to 60° for twenty minutes renders the milk harmless, not only so far as concerns tuberculosis, but also in destroying the infectious agency of cholera, typhoid and diphtheria.

H. W. C.

**Dorset.** The Use of Eggs as a Medium for the Cultivation of the tubercle bacilli. American Med. 3: 555, 1892.

The method adopted by the author is to mix together the white and the yolk of eggs in test tubes and then, by keeping the material in the tubes at 70° C. for four hours upon two successive days, it is both sterilized and coagulated. The use of the yolk alone does not produce a very abundant growth of the tubercle bacilli and the use of the white alone is quite unsatisfactory. A couple of drops of sterilized water is placed in each of the tubes to moisten the material, and then the tuberculous matter is inoculated upon the surface of the medium. An abundant and satisfactory growth has been obtained from tuberculous material from guinea pigs.

H. W. C.

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Ever since the JOURNAL has been published, but more particularly during the past year, we have had inquiries for practical methods for the microscopical examination of adulterated foods and other commercial products, and for methods of determining, by microscopical examination, the composition of commercial products, such, for example, as paper, textile substances, paints, etc. We have communicated with many of the directors of laboratories in which microscopical work is done in the leading institutions of our country, and have, almost without exception, found that not only was there no attention paid to such subjects in these laboratories, but the laboratories could furnish no information as to where such work was being done, in fact, were entirely out of touch of the practical application of microscopical work in the industries. While we do not wish at this time to question the good which is being done in the biological laboratories of our country in microscopical investigations of biological subjects, we do think it is time that a public sentiment should be created which will cause the directors of our laboratories to give at least some attention to the practical application of the microscope in industrial work. Papers are made from an infinite variety of substances, and the cost of production and their commercial value depend upon the materials of which they are composed to a greater extent than upon the labor consumed. This fact offers a fertile means for the manufacturer and dealer to obtain a higher price for a low grade article, and the only protection that the consumer has is in knowing just what the composition of the paper is.

The laundry business of our country is of enormous extent. Starch is one of the large elements of expense in the conduct of a laundry. Corn starch and wheat starch are sold for laundry purposes. Wheat starch is the better of the two, and is very much higher in price. A mixture of a certain percentage of corn with wheat starch gives a starch which is absolutely undetectable from pure wheat starch, as far as its use in the laundry is concerned, and this mixture is very largely used. The relative quantity of corn and wheat starch should determine the price, but here again the consumer must rely entirely upon the statement of the seller, unless he is provided with a microscope, and the knowledge by which he can determine the relative proportions for himself. Other starches besides corn starch are used in adulterating laundry starches, and their presence and amount can only be detected in this way. There are hundreds of other uses for the microscope in the industries.

We should like to be contradicted in our statements, and to have the contradictions substantiated by the placing before our readers of some of the methods which have been evolved in the various laboratories of the country for these purposes.

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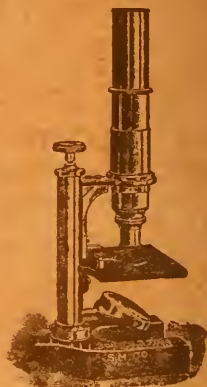
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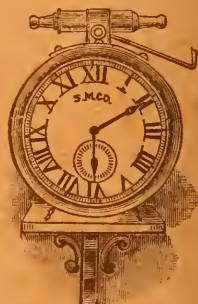
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# Journal of Applied Microscopy and Laboratory Methods

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## On Embedding in Celloidin.

In cutting sections of tissues which have been embedded in celloidin according to the method now in general use, certain difficulties are often met, which are due partly to inaccurate preparation of the solutions of different strengths, partly to insufficient infiltration owing to leaving the tissues too short a time in these solutions. Thus, for example, in cutting portions of the alimentary tract, one is frequently forced to cut thicker sections than he would like, owing to the fact that the sections tear apart in the tela submucosa. By the use of the following method many disadvantages may be overcome, and I advise its use in all those cases in which accuracy of results is of sufficient importance to justify the greater expenditure of time and care.

Very satisfactory results were obtained with this method with nerve tissue that had been in Müller's fluid several years. By the old method it was very often necessary to fortify the sections to keep them from crumbling and breaking, but the tissues are so thoroughly penetrated by this method, that I have stained by Weigert's method sections cut at  $10\mu$  without taking any additional precautions to keep the sections intact.

Schering's granulated celloidin, or better, the moist cake celloidin is used. In the latter case, the cake is cut into thin strips and allowed to dry in a glass dish protected from the dust. When dry, it has a yellowish tint, and is very hard. One cake of Schering's celloidin when dry weighs from 28 to 30 grammes. Ten wide-mouthed, cork-stopped bottles should be cleaned and *thoroughly dried* for the celloidins. The different solutions are made up so that each 100 c. c. contains two, four, six, etc., up to twenty grammes by weight of celloidin. Tissue that has been thoroughly dehydrated, and passed through absolute alcohol and ether, is passed through the graded celloidins, being left twenty-four hours in each solution. If to be cut immediately, the tissue is mounted on a block and hardened in chloroform for from fifteen to twenty minutes, or in eighty per cent. alcohol for several hours. If it is to be kept for some time, the tissue should be removed from the twenty per cent. celloidin with a thick layer of celloidin surrounding it, and dropped into chloroform to harden, after which it is kept in equal parts of ninety-five per cent. alcohol and glycerine.

When wanted for cutting, the tissue is wiped dry with a clean cloth, a thin layer of celloidin is shaved off, and the piece immersed in six per cent. celloidin several minutes, then mounted on a block, and hardened in chloroform.

The only disadvantage of the method is the time taken to completely embed an object, at least twelve days, but one is amply compensated for this by the many advantages which the method affords. Among these may be mentioned the following:

I. Many tissues which would suffer shrinkage if transferred abruptly from thin to thick celloidin, may be embedded without injury.

II. Owing to the gradual method of advancing from weaker to stronger solutions, the ultimate concentration of the solutions may be increased much beyond that ordinarily employed.

III. More perfect infiltration of the object is attained.

IV. The resulting block of celloidin is of firmer and more uniform consistence, enabling one to cut much thinner sections, without tearing or separation.

V. The preservation of blocks of celloidin in alcohol glycerine permits of the storage of large quantities of class material in much smaller space than would be required if each piece were mounted separately on a fibre block. Moreover, this method admits of the trimming of the celloidin blocks after hardening in chloroform, thus saving a great deal of celloidin for subsequent use.

VI. With several blocks of compressed fibre for mounting, one can do a large amount of work.

I have found the following method very convenient for keeping a record of tissues.

Each piece of tissue on removal from the animal is entered in a book by number, together with name of animal and fixation. On taking each piece from the twenty per cent. celloidin, a small tag of stiff paper, with number in lead pencil, is attached, and embedded with it, and the whole dropped into chloroform to harden. The tissue is then preserved in alcohol glycerine until wanted.

Hull Laboratory of Anatomy, University of Chicago.

CHARLES H. MILLER.

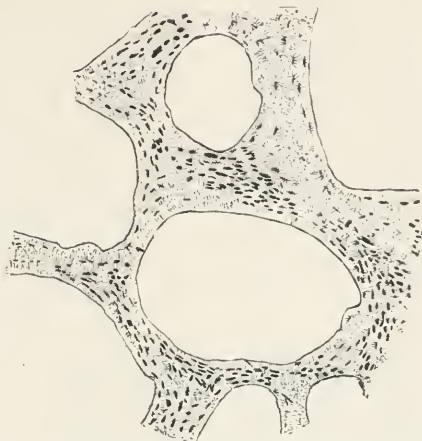
## A Method for Preparing Sections of Cancellous Bone.

Much difficulty is experienced in grinding sections of dry bone and tooth (especially of cancellous bone) by the usual method of embedding the material in cork or holding on the grindstone with the finger. By these methods it is very difficult to grind all parts of a section to the same thickness, the piece of material cannot be held firmly in one position; thus the soft parts of the tissue are ground thin before the more compact, and a section in the desired direction (as a direct transverse section, longitudinal section, etc.) is difficult to secure.

The following method has proved very satisfactory in preparing sections of bone, tooth, shell, and especially cancellous or spongy bone:

With a fine saw cut sections (about one-half mm. in thickness) of cancellous bone tissue from a well prepared piece of bone, bone which has been macerated until all fatty substance is removed, then allowed to dry. Rub the section on a

whetstone until smooth on one side, place this side downward on a slide; cover the section with Canada balsam dissolved in xylol, ignite the balsam and allow it to burn as long as possible without injury to the section of bone; then extinguish and press the section firmly to the slide until the balsam hardens. This method of freeing the balsam from xylol gives better results than heating the slide over a flame, as it does not allow the balsam to spread. The section of bone, now firmly embedded in the balsam, can be held on a small grindstone by holding the ends of the slide, then rubbed down on a whetstone to any desired thickness. From



A camera drawing of cancellous bone, from section prepared by embedding in Canada balsam.

time to time during the process of grinding, the slide may be placed under a low power of the microscope and the thickness of the section noted. When the structure of the bone is plainly visible, clean slide and section with damp cloth, dry thoroughly, add drop or two more of balsam and cover-glass.

The accompanying drawing was taken from a section of human bone prepared by this method.

E. O. LITTLE.

DePauw University.

## A New Agent for Use in Tide Pool Collecting.

During the fall of 1900, in the course of a series of experiments made for the U. S. Fish Commission by the writer, to determine the effect of sulphuric acid and its salts on the Sacramento salmon, it was noted that copper sulphate is peculiarly poisonous to aquatic organisms. This substance in solution 1:1,000,000 usually caused death of the fish within twenty-four hours, and stronger solutions proportionately earlier. In view of this fact, the use of the salt in tide pool collecting was suggested by Mr. R. C. McGregor. Experience showed that, owing probably to the carbonates in sea-water (or, possibly, to the formation of a hydrate) an opaque precipitate is formed that interferes seriously with its use. This has been obviated at the suggestion of Mr. Loye Miller by the addition of a few ounces of crude sulphuric acid to each gallon of saturated solution of the bluestone. The salt is soluble at ordinary temperatures in about twice its weight of water. A quart of this prepared solution, costing about ten cents, will poison a pool containing 100 cu. ft. of water.

The more readily migratory forms, as eels, leaping fish, and hermit-crabs, show the first result by their attempts to escape. In this dilution the copper kills only the smaller fishes, labroids and butterfly fish being among the more susceptible. Buried worms and similar forms emerge after a time. The greater

number of specimens will, however, be taken by means of a dip-net as they come to the surface for air or swim across the pool in search of a means of exit. In pools in honeycombed coral rock or other tunneled material, often the most fruitful time is just as the tide begins to enter and force the poisoned water into the tunnels.

While possibly possessing no superiority over calcium chloride for this purpose, it is worthy of note as an additional agent at the disposal of the collector.

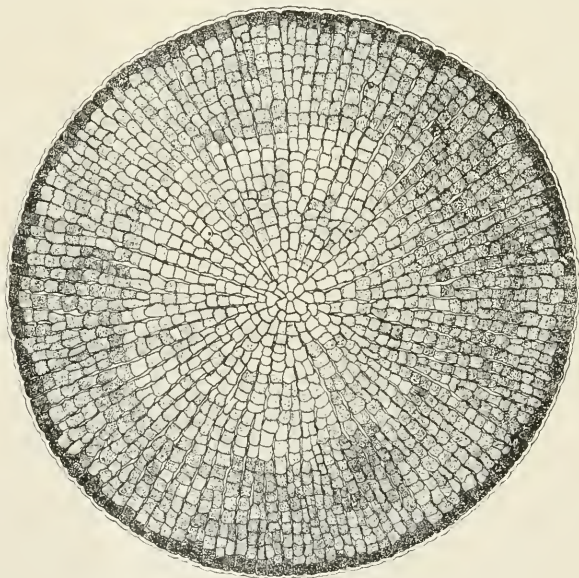
U. S. Fish Commission, San Francisco.

F. M. CHAMBERLAIN.

### A Method for Raising Coleochaete.

One of the greatest difficulties to be overcome in the laboratory is found in trying to obtain cultures of some of the algæ.

For a number of years we were unable to secure a culture of *Coleochaete*. However, in September of 1899, in making our collection of algæ for winter use, material gathered from a small pond in the vicinity was placed in a battery jar of hydrant water. The mouth of the jar was covered with a glass plate and sealed with vaseline. The jar was then placed in a south window.



*Coleochaete orbicularis*. Camera lucida drawing.

At different times during the winter various forms of alga were found growing in the culture. In the latter part of March the *Coleochaete orbicularis* was discovered to be growing in great abundance around on the inside of the jar.

This last fall a culture of *Zygnema* was taken from a pond and placed in an open jar of hydrant water. The *Zygnema* degenerated, but by the last of March, as in 1899, the *Coleochaete orbicularis* appeared in large numbers, and the individual plants were larger in size than those of the previous culture.

Judging from these two results it seems probable that *Coleochaete* may be obtained for laboratory work by this method.

MARY AVIS HICKMAN.

Biological Laboratory of De Pauw University.



## Laboratory Notes.

### I.

**A Simple and Effectual Method for Keeping Media and Cultures Sealed.**—Repeated efforts to devise some means of keeping media and cultures hermetically sealed resulted in the following device: Paraffin stoppers are made from paraffin of the highest melting point ( $71^{\circ}\text{C}.$ ). These are kept in a solution, containing 50 per cent. of alcohol and 3 per cent. of copper sulphate, until used. The stoppers are of various sizes to fit the various sized test-tubes (Fig. 1-*c*). To seal the tube a size somewhat larger than the tube is selected, the cotton plug is thoroughly singed and inserted to within  $\frac{1}{2}$ -inch from the end. The latter is warmed and the stopper inserted. The tube then has the appearance of Fig. 1-*b*. When required for inoculation, the end of the tube is gently warmed and the stopper removed. It may then be held between the fingers during inoculation and replaced, or it may be rejected and placed back into the solution, and the cotton plug alone used. The advantage of this method over rubber caps is, first, in the cheapness and, second, freedom from molds, as the small amount of copper sulphate-alcohol adhering to the stopper is sufficient to kill the molds on the upper end of the cotton plug. The advantage over a paraffined plug is that it is less greasy and leaves the cotton plug dry and expansile. Solid media in tubes with paraffined plugs cannot be melted without the paraffin running down into the medium and spoiling it for plate cultures. This is entirely avoided by the use of the stopper.

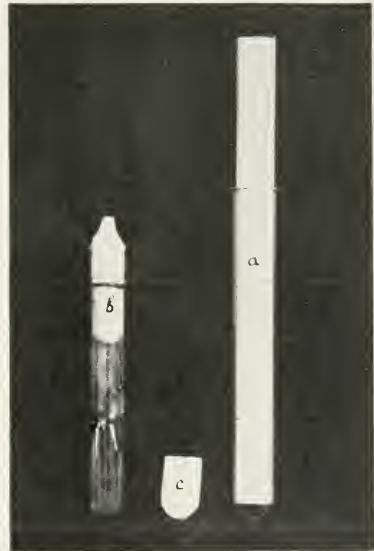


FIG. 1.—(*a*) Paper mould in test-tube; (*b*) agar-agar slant with plug and paraffin stopper; (*c*) paraffin stopper.

To make the paraffin stoppers, the following method is employed: A strip of ordinary stiff writing paper is rolled into a tube and inserted into a test-tube (Fig. 1-*a*). By rotating the paper tube, it is so adjusted as to fit the test-tube snugly. Melted paraffin is then poured into the paper tube and the whole at once placed in running cold water. When solidified the walls of the test-tube are slightly warmed and the inner paper mould removed by a slight rotatory movement. The paper is then easily peeled off from the solid paraffin stick. This is cut up into cylinders of desired lengths and the latter trimmed into shape with a warm knife. The whole procedure takes but a few minutes.

### II.

**The Preparation of Loeffler's Blood Serum Plates for Diphtheria Bacilli.**—In the preparation of Loeffler's blood serum we found the method first proposed by Miss

Carter (*JOUR. APP. MIC.*, April, 1898) and subsequently recommended by Dawson (*JOUR. APP. MIC.*, Oct., 1899) most satisfactory. The surprise is that it is not generally recommended in text-books. It is certainly admirably adapted,



FIG. 2.—Flask, plugged and stoppered, containing coagulated blood-serum and showing colonies of diphtheria bacilli.

especially to class work. One point about getting perfectly white serum. The usual recommendation of using the serum after it has stood for 24 to 48 hours does not give satisfactory results, as the serum will always be somewhat colored. A better practice is to put the serum drawn off at the end of 48 hours into a well-stoppered bottle, add a little chloroform and let it stand for weeks and even months. In time, the blood corpuscles subside, leaving a perfectly clear serum. This, when coagulated, is as white as the white of egg. The chloroform is removed by heating the serum to be used at a  $56^{\circ}\text{C}$ . for 30 minutes. For the isolation of diphtheria bacilli the following method will prove fairly satisfactory. Round flat flasks, containing Loeffler's blood serum mixture, are placed in the coagulator or sterilizer on a perfectly horizontal plane, and the serum coagulated. It then presents a perfectly white smooth surface. The swab is run lightly over this surface, and the

flasks inoculated, bottom side up, for 18 to 24 hours. At the end of that time any colonies which have appeared on the surface are removed by means of a platinum needle with the point bent at a right angle and examined. The minute colonies which appear at this time of incubation are, as a rule, either micrococci or diphtheria bacilli. By examining several colonies the right colony may be finally discovered. The reason for using flasks instead of plates, although the latter are more convenient, is that the flasks can be hermetically sealed by means of the paraffin stoppers and kept ready for use. (See Fig. 2.)

### III.

**A Ready Method for Manipulating and Fastening Paraffin Sections.**—The following method will be appreciated by those who exhaust their patience on trying to handle and fasten to the slide paraffin sections: The paraffin sections are floated in warm water. The center of the slide is painted with a saturated solution of celloidin in oil of cloves. With the corner of a piece of filter paper one or more of the sections are lifted out by placing the paper underneath the sections. The latter adhere by capillary attraction to the filter paper. The paper holding the sections is then inverted over the celloidin-oil and gently pressed. The sections adhere firmly to the slide, and the paper can be readily removed without carrying the sections along. The subsequent treatment is the one usually recommended. By making a rather thick film of celloidin-oil and then

removing the paraffin from the sections as well as the oil of cloves by means of xylol, it may be possible to obtain satisfactory celloidin mounts which can be gently detached from the slide and treated as celloidin sections.

#### IV.

**A Precaution as to the Use of Fuchsin for Staining Tubercle Bacilli.**—In the course of our routine examination of sputum for tubercle bacilli we had occasion to use by mistake carbol-fuchsin prepared from acid-fuchsin or “Fuchsin S;” Gruebler. We found that the tubercle bacilli, when present, are but faintly stained; that other bacteria retain the stain after decolorization; and that when the specimen is counterstained, the tubercle bacilli are readily overlooked. This fact had been established by making comparative preparations stained with acid fuchsin and basic fuchsin. The precaution is that in making carbol-fuchsin the basic dye alone should be used.

A. ROBIN.

Delaware State Board of Health Laboratory, Newark, Del.

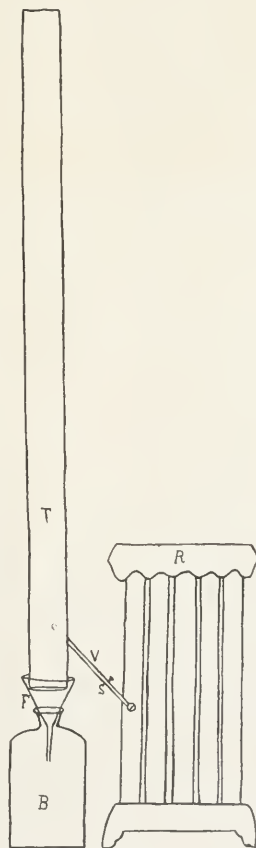
#### A Still.

The accompanying illustration shows a very simple apparatus for securing large quantities of distilled water in laboratories heated by steam. With the apparatus as shown in the diagram, distilled water may be secured at the rate of from fifteen hundred to three thousand c. c. per hour. The cost of the apparatus need not exceed one dollar. A piece of metal tubing, V, one-half inch in diameter and from twelve to eighteen inches in length, is connected with the air-valve or vent of a radiator, R. A piece of tin spouting, T, about five inches in diameter and six feet or more in length. The piece of spouting is placed in an upright position and the tube V from the radiator is passed into it through a small opening near the lower end. The upper end of T is closed by a flat piece of tin, and by the valve S the amount of steam allowed to pass from the radiator into T can be regulated; no more steam should be allowed to pass into the tube than will be condensed. The water is collected by means of the glass funnel and bottle, F and B.

The apparatus as described has been in use for two years, and has given excellent results.

DePauw University.

E. O. LITTLE.



A Simple Still.

## A Review of the Methods of Staining Blood.

### VII.

#### D. *Neutral Stains*.—Continued.

*Willebrand* (1901) and *Becker* (1901) obtained a neutral stain by adding acetic acid to a mixture of eosin and methylen blue. In a mixture of eosin and methylen blue diffuse staining is obtained, but if dilute acetic acid be added drop by drop, a differentiation takes place. *Becker* believes that the acetic acid acts as a solvent of the neutral precipitate.

Equal volumes of a 0.5 per cent. solution of eosin in 70 per cent. alcohol and of a concentrated watery solution of methylen blue are mixed. To 50 c. c. of this mixture ten to fifteen drops of a 1 per cent. acetic acid solution is added. The solution should be filtered before using. Preparations which have been fixed by dry heat, absolute alcohol or 1 per cent. formol alcohol, should be stained five to ten minutes, washed in water without decolorization. Red corpuscles stain red, nuclei dark blue, acidophile granules red, neutrophile granules violet, and granules of the mast cells intense blue.

*Ewing* (1901) also uses acetic acid as a solvent of the neutral dye.

1. To 30 c. c. polychrome methylen blue (*Grübler*) add five drops of a 3 per cent. solution of acetic acid (V. S. P. 33 per cent.).

2. Make a saturated 1 per cent. aqueous solution of methylen blue (*Grübler*), dissolve dye by gentle heat. This solution should be at least a week old.

3. Make a 1 per cent. aqueous solution of *Grübler's* aqueous eosin.

To 10 c. c. of water add four drops of the eosin solution, six drops of the polychrome methylen blue solution, and two drops of the 1 per cent. methylen blue solution. Mix well. Preparations should be stained two hours, blood side down.

Several hematologists have obtained a neutral staining by successive staining with eosin and methylen blue, the neutral stain being precipitated in the protoplasm of the cells.

*Engel* (1901) stained fixed dry preparations five minutes in an eosin solution (eosin 1 g., water 90 g., alcohol 10 g.), washed in water and stained thirty seconds in a concentrated watery solution of methylen blue or *Löffler's* alkaline methylen blue. It is important that the methylen blue should not act too long on the preparation. All of the basophile, acidophile and neutrophile elements of the blood are stained. *Engel* thinks that failure to get a differentiation of the basophile, acidophile and neutrophile elements by successive staining with eosin and methylen blue is due to too long action of the methylen blue. He believes that a previous preparation of a neutral dye is unnecessary.

*Japha* (1901) also stained successively with eosin and methylen blue. He fixed dry preparations for one minute in 1 to 2 per cent. formalin in alcohol, stained well with a strong watery solution of eosin, then several seconds with dilute—but not too dilute—watery solution of methylen blue. Judgment must be used in staining with the methylen blue. *Japha* examined the washed preparation from time to time to determine the degree of staining.



*Goldhorn* (1901) fixed air-dried blood preparations for fifteen seconds in pure methylic alcohol or in pyroxylic spirits, washed in running water, stained for seven or more seconds in  $\frac{1}{10}$  to  $\frac{1}{5}$  per cent. aqueous solution of eosin (preferably Grüber's yellowish *wasserlöslich*), washed and again stained for ten seconds in a faintly alkaline solution of his polychrome methylen blue, which is prepared as follows: Dissolve two grams of methylen blue in 300 c. c. of water. Shake the solution well and add four grams of lithium carbonate; shake again thoroughly, and pour into a large open porcelain dish over a water bath. Allow the boiling water to touch the bottom of the dish. Stir frequently and decant, after fifteen minutes, into a glass-stoppered bottle. Filter paper should be avoided, cotton may be used in the funnel. Set aside for a few days and slightly acidify with a 4 or 5 per cent. solution of acetic acid. Now add enough of a saturated solution of lithium carbonate to render the stain faintly alkaline. In order to know when this point has been reached trial blood smears should be stained until the addition of another trace of lithium carbonate causes the red corpuscles to take some of the blue. With some practice this point is rapidly determined. A stain prepared in this manner does not deteriorate, but on the contrary seems to improve on keeping if kept in well-stopped bottles. To determine the best time of staining, a number of preparations should be left in the dye for a varying number of seconds and the interval giving the desired result fixed upon.

Nuclei of the megaloblasts stain beautifully, an intranuclear net-work is well shown. Necrosis and polychromatophilia of the red cells are shown. Other nucleated red cells stain well. Polymorphonuclear neutrophils show their granules and "chromatin-bridges." The lymphocytes stained for a short time frequently show nucleoli in a thick capsule, and basophile granules in the cytoplasm. The lymphocytes may be stained deeply, hence the value of the stain in lymphatic leukaemia. Blood plates show purplish dots within a bluish body.

Goldhorn's polychrome methylen blue, improved formula, is now for sale, ready for use. The staining technique is as follows:

Methyl alcohol, fifteen seconds, wash in running water.

Eosin,  $\frac{1}{10}$  per cent. aqueous solution, seven to fifteen seconds, wash as before.

Goldhorn's polychrome methylen blue, thirty to sixty seconds. Wash thoroughly; dry by agitation of the smear in the air, avoiding heat and the use of filter paper. Mount in Canada balsam.

Should the polychrome dye by exposure to the air become too alkaline—the erythrocytes staining too deeply—add a few drops of a 4 or 5 per cent. solution of acetic acid. The stain does not deteriorate, but improves on keeping.

For staining malarial parasites it is recommended that the polychrome dye should act for from 90 to 120 seconds, that the chromatin of the half-grown forms may become well stained.

*Hanna* (1901) describes a modification of the Romanowsky-Nocht method, given by Berestneff (in Russian). A 1 per cent. aqueous solution of methylen blue (med. puriss. Höchst), containing 0.3 per cent. of carbonate of soda, is heated for three hours on a water bath and then filtered. One c. c. of this solu-

tion is mixed with 1.5 c. c. of a 1 per cent. aqueous solution of methylen blue, and to this mixture is added 5 c. c. of a 1 per cent. aqueous solution of eosin (extra Höchst).

Fresh preparations of blood fixed in absolute alcohol are stained in this mixture, diluted with from two to four volumes of water for five minutes, without heat, followed by gentle heating for from five to ten minutes. To decolorize the red cells, which are blue after this staining, the preparation is put for from two to five seconds in the following mixture: 10 c. c. methylen blue, 1 per cent.; 200 c. c. distilled water, and 0.25 c. c. acetic acid. The preparation is then washed in water, dried with blotting paper, and then dipped for five to twenty seconds in absolute alcohol to dissolve out the residuum of stain, and finally washed in water.

Red corpuscles stain a delicate rose color, acidophile granules purple, neutrophile granules rose color, nuclei and Bizzozero's plates bright red violet, protoplasm of malarial parasites blue, chromatin of malarial parasites bright red violet.

ERNEST L. WALKER.

Massachusetts State Board of Health.

## The Museum.

### V.

#### INTERIOR PLAN—Continued.

Some general dimensions for the halls of a Science and Art Museum, if conforming to the standard of a many storied building for the former and a single or double storied building for the latter, may be resumed in the following directions:

#### SCIENCE MUSEUM.

Whole length of separate hall sections, 180 to 220 feet.

Width of separate hall sections, 60 to 65 feet.

Five Stories,	-	-	Height.	Six Stories,	-	-	Height.
1st floor	-	-	20 to 24 ft.	-	-	-	18 to 24 ft.
2d "	-	-	22 to 25 ft.	-	-	-	22 to 28 ft.
3d " (gallery)	-		15 ft.	-	-	-	15 to 17 ft.
3d " (not as a gallery)			18 ft.	-	-	-	18 ft.
4th "	-	-	22 ft.	-	-	-	18 ft.
5th " (work rooms)			15 ft.	-	-	-	12 ft.
				6th floor	-	-	12 ft.

Terminal or central halls, which may be embedded between the larger units (viz., the standard halls), 60 to 70 x 100 to 130 feet. Tower spaces, arbitrary. In cruciform structures, a *Rotunda*, etc., unites the arms.

#### ART MUSEUM.

Central skylighted court, 150 x 100 x 75 feet (latter dimension to roof or top of arch, if spanned). Surrounding hall, 30 to 50 feet wide, divided by arches, columned doorways, etc., into suitable minor compartments or halls according to

nature of exhibits. The outer hall may be doubled, forming two enclosing *boxes* to the court. In the latter case there may be loss of light in the inner halls.

#### SCIENCE MUSEUM—One Story.

Of this style of building the Field Museum may be taken as a quite successful illustration. The ground plan (Fig. 20) of the Chicago museum is cruciform, made up of two main halls, crossing under a rotunda, and a number of smaller tributary halls at right angles to the east and west main hall, an arrangement,

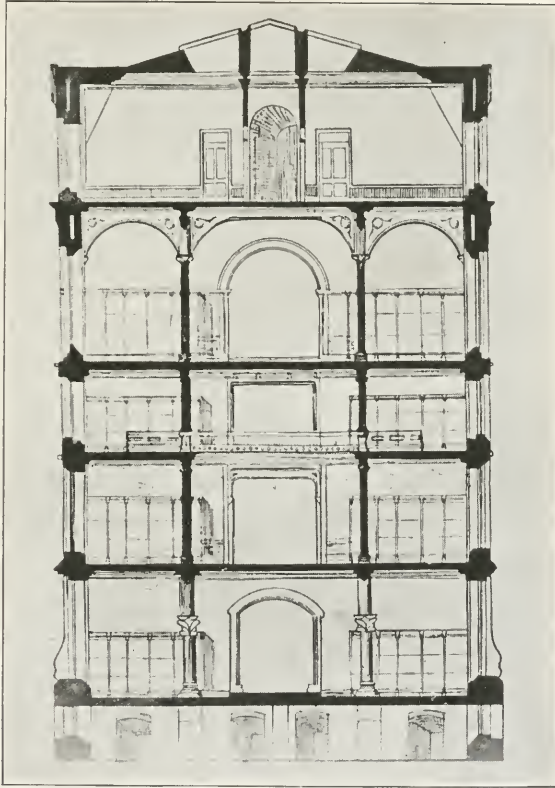


FIG. 21.—Vertical Section of American Museum of Natural History, New York.

according to the thesis maintained here, generally objectionable; in fact it may be claimed that the single-story Science museum should be abandoned.

The main arms of the cross in the Field museum are 99 feet wide, 69 feet high, and respectively 300 and 501 feet long, with the central dome 126 feet high.

Examples of interior sections which furnish some useful comparisons are given in Figs. 21, 22, 23. Fig. 21 is a vertical section through the first member of the American Museum of Natural History, New York; Fig. 22, the same through the Academy of Sciences of Chicago; and Fig. 23, the same through the Art Institute in Chicago, in which for scientific utility the superiority of the first seems incontestable, though the second is conceived on similar lines. The dis-

position of the gallery floor at a lower level, as in the New York museum, is to be preferred, as it allows an extra exhibition floor, and permits the use of the top floor for offices, laboratories, etc. The Chicago Academy of Science possesses an admirable building, well illuminated, with an interior most intelligently equipped, and it can be readily admitted that it conforms generally to ideal standards.

The construction of the museum building demands the most advanced safeguards against fire. This need hardly be insisted on. The melancholy recollection of the fires which destroyed two collections of the Academy of Science,

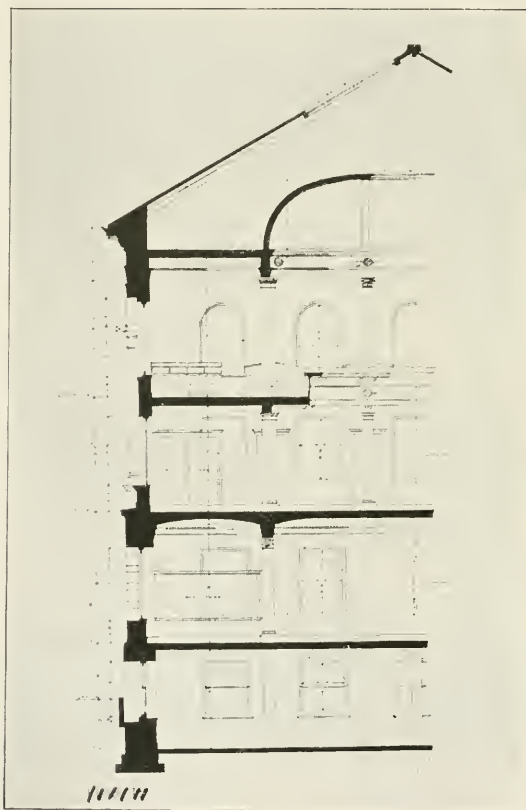


FIG. 22. —Vertical Section through Academy of Sciences, Chicago.

and that of the New York Academy, the fires which destroy the art treasures of private collectors, emphasize the recurrent need of enjoining upon builders of museums extreme caution.<sup>1</sup>

In this connection the only suggestion that is paramount, is the use of public squares, parks, and reservations, where the proximity of houses and buildings subject to daily risks is avoided.

<sup>1</sup> The builders of museums are recommended to the publications of the British Fire Prevention Co., Pall Mall, London, for useful and comprehensive discussions of fire and its prevention.



Emphasis is also to be laid upon the protection of walls from exterior dampness; the defacement of walls, by the infiltration of water, adds to the expenses of maintenance, not infrequently is injurious to exhibits, and complicates labor by compelling the movement or reconstruction of wall cases. The air space in the double wall, with both walls of the air cavity painted with tar, forms perhaps the most complete defense against dampness. Such preservation in regions visited by heavy storms, characterized by a wet climate, and exposed to oceanic influences, is of very grave importance.

Protection against dust merits serious consideration. The wood of sashes

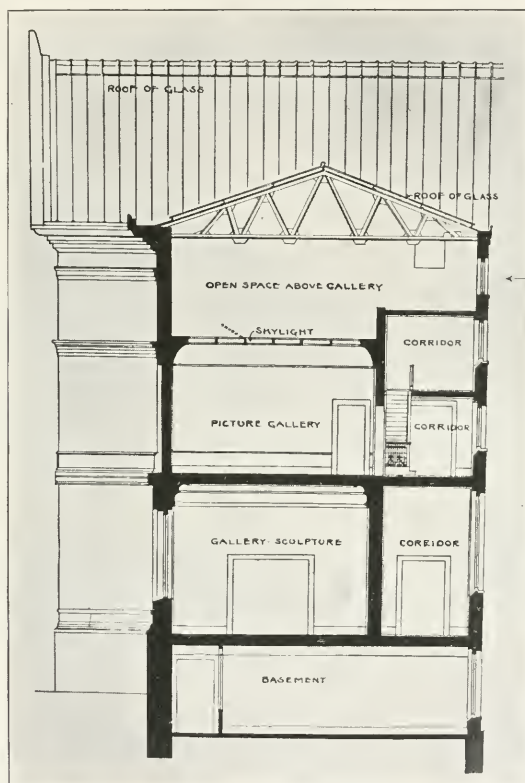


FIG. 23.—Vertical Section through Art Institute, Chicago.

should be seasoned and past shrinkage, the runways of the window frames should be *deep*, and the union of the double sashes in windows well secured by *two* locks. Double windows can only be doubtfully recommended. Dust is a variable accompaniment of position. It is more grievous naturally in dirty cities than in clean ones, in tropical positions than in boreal, and on unpaved roads and streets than where roads are properly paved, curbed and drained. The prevalence of dust in the warmer seasons, when the windows of halls are unavoidably open, can be sensibly diminished by keeping the neighboring streets well sprinkled. And it will generally be found that proper representation,

of the urgency and value of the protection, asked for, to municipal authorities will secure for the museum this desirable concession.

In this section on the Interior of the Museum a few words may be profitably devoted to the subject of interior ornament. Many will recall the suggestive symbolism of the Pavilion of the Fish Commission at the Chicago World's Fair, and the mention of ornament, mural, decorative, and architectural, will remind the visitors of the Natural History Museum, London, while the more pretentious introduction of ornament is seen in the Vienna museums.

A sweeping prohibition of all such adventitious accessories is desirable. What advantage is gained by these rudimentary effigies of natural objects on walls and columns, friezes and pedestals? Even the larger and more serious use of mural painting, presumably, under ordinary circumstances, should also be discouraged.

The interior of a museum should be the most easily subjected to all cleansing processes, and processes of renewal as well. A variety of ornament furnishes nests, retreats and skulking corners for dust and dirt, and painted walls are so easily defaced that the avoidance of this sort of illustration is commendable. A grace of architectural structure with *simple surfaces* is alone to be aimed at. This disposition is pleasing to the eye, and admits of renewal and cleansing, and almost inevitably precludes expense in maintenance.

Finally, ventilation is most important, and a system which may preclude the opening of windows, or demands only a minimum of such aeration, should be installed in large museums. The double window in use on the Continent is generally useless. As Dr. Meyer points out, it interferes with the light, and in cold weather they form an outer screen of frosted panes. It seems feasible, under the most arctic conditions, to keep halls warm with single windows; the resources of modern art are ample enough for the most severe exactions in this respect.

The flooring of museums is preferably made of tiling than of wood. The former can be cleaned and washed with care, and dust thus sensibly eliminated. The latter is itself a source of dust, wearing away by the attrition of use into a fine powder; it is not always successfully cleaned, and retains moisture after mopping.

In styles of tiling there seems to be no especial reason for choice. The small square blocks of the Pompeian tile seem durable and certainly in art museums have an artistic congruity that is attractive. The larger squares of the encaustic tile are also excellent, but, as a matter of good taste, they should not be too varied in color, nor should patterns of much complexity be adopted. Simplicity is usually a safe guide. Three colors are used with good effect, a buff and black and red. There is needed a certain forcefulness and beauty in the tiling of a great handsome hall, but the limits of moderation may be easily overstepped. There is no necessity of strict uniformity in tiling throughout a museum; the different floors and even the different halls may vary, as the carpeting of a house may vary from room to room, but pretentious contrasts are of course objectionable. Foreign tiles seem generally more durable, and less absorbent of water.

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American Museum of Natural History.

(For many figures illustrating the sections, so far published, of this paper, the author is indebted to the Memoirs of Dr. A. B. Meyer, of Dresden.)

## Methods in Plant Physiology.

### X.

#### XI. GEOTROPISM.

In selecting material for experimentation in geotropism care should be taken to use only vigorous, healthy plants; no credence can be given to results obtained from unhealthy or dwarfed plants. When the roots of seedlings are to be used they should be grown in moss or clean pine sawdust. If the sawdust becomes infested with fungi a new supply should be obtained. All potted plants used in this work should be, if possible, grown out of doors, failing of this, the supply may be obtained from a greenhouse, but such material is not as reliable. The light must be carefully excluded from all experiments. While preparing the plant for experimentation it is essential that it be kept in the vertical position until ready

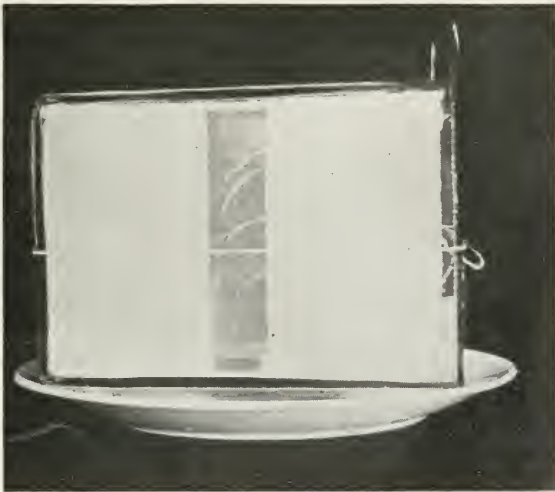


FIG. 11.—Damp Chamber used in Geotropism.

to apply the desired stimulus. If the results of two or more experiments are to be compared, the temperature at which the experiments are to be performed must be the same in every case.

1. **Region of Curvature in Roots.** Select seedlings of any species having a large, straight main root, from 2–5 c. m. in length, e. g., the lupine (*Lupinus albus*) or the pea (*Pisum sativum*). Beginning at the apex mark the root into millimeter spaces for a distance of one centimeter with dots of water-proof India ink. Fasten the seedlings with rubber bands and strips of blotting paper to a bar of wood and suspend them in a small damp chamber. Leave a strip of the surface of the jar uncovered by the paper in such a way that observations may be made without disturbing the seedlings. (Fig. 11.) Arrange the seedlings so that the ink marks may be seen through the window in the jar. Fasten the cover and lay the preparation on its side in a plate which contains enough water to keep

the inside of the chamber moist. In this position the seedlings are exactly horizontal.

Incipient curvature may be observed at the end of 3–5 hours and the retrogression of the curve during the next 10–24 hours. This will also furnish a means of comparing the region of curvature with the region of growth.

2. **Region of Curvature in Unjointed Stems.** Potted plants or cut stems of *Vinca major* are convenient to use for this experiment. If the latter is selected cut off the stem under water, and pass it through a hole in the cork of a bottle of water, packing the stem firmly with cotton batting. Mark the terminal three or four internodes at intervals of 5 mm. and place the stem in a horizontal position. Make observations as in the previous experiment.

3. **Region of Curvature in Jointed Stems.** For this experiment use stems of *Tradescantia* or of some grass. Remove all the leaves and prepare them in the same manner as in the preceding experiment, except that the stems are marked off at intervals of 2 mm. and two rows of dots are made opposite each other.



FIG. 12.—A Root growing into Mercury.

When the stem is placed in the horizontal position one row of dots should be directly below the other.

This experiment affords a convenient means of demonstrating that these responses are growth curvatures and that the response is manifested only in the growing zone. The marks on the dorsal and ventral sides of the stem show the relative amount of growth on the different sides.

4. **The Position of Greatest Stimulation.** The fact that plants respond differently to different degrees of stimulation may be shown by preparing roots as in Experiment 1, and inclining them at angles of  $45^\circ$ ,  $90^\circ$ ,  $135^\circ$ , and  $180^\circ$  from their normal position. The angle at which the response is first manifested should be carefully noted.

5. **Latent Period.** The latent period which elapses between the application of the stimulus and the first response is satisfactorily exhibited by nearly all growing stems. A potted plant or a crock of seedlings growing in earth give the best results. Thrust two slender iron wires into the earth on either side of the stem, giving them the same direction and height as the stem. Turn the



plant over so that the stem and wires shall be in the same horizontal plane. Make observations at intervals of 15 minutes to determine the time when the stem begins to move out of its place. Record the temperature.

6. **After Effect.** When the plant used in the preceding experiment has acquired a curve of  $10^{\circ}$ – $20^{\circ}$  revolve it  $180^{\circ}$  from its former position and immediately bend the wires to conform to the curve of the stem. Observe at intervals of 10–15 minutes to observe how long the plant continues to bend in the original direction. The temperature should be the same as in the foregoing experiment. A more exact method of determining the after effect is by using the roots of seedlings. When they have been exposed to the stimulus of gravitation, as in Experiment 1, long enough to cause pronounced curves, they should be transferred to a klinostat and revolved in such a way that gravitation will be neutral-

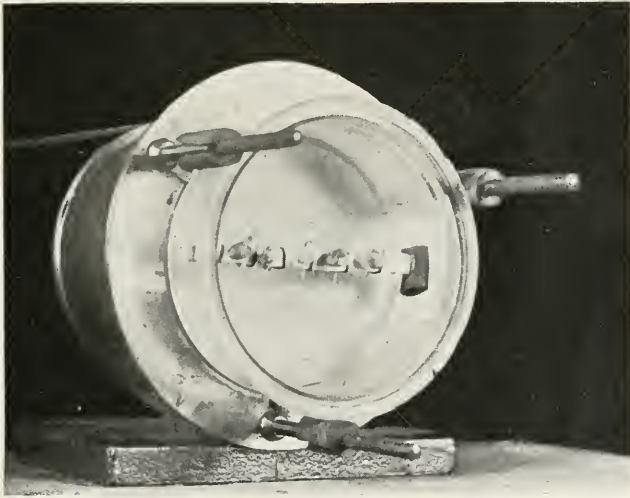


FIG. 13.—A damp chamber in use on a klinostat. The power is furnished by a small electric motor. The speed is reduced by the intervention of a worm-gear.

ized. Observations may be made every 10 minutes to determine the length of time the root continues to curve.

7. **Force Exerted by Growing Roots.** A satisfactory means of demonstrating that the downward growth of roots is an active, not merely a passive, response, due to the gravity of the root, is shown in Fig. 12. A lupine or *Vicia Faba* seedling is placed in a horizontal position in a large damp chamber; immediately beneath the tip of the root is a small dish containing washed mercury to a depth of 1 to 2 c. m. When the root curves downward it penetrates the mercury but is not forced into it by the mere elongation of the root-tissue.

8. **Simulation of Gravitation.** *Knight's Experiment.* Fig. 13 represents a convenient means of performing this classic experiment by the use of an ordinary damp chamber which can be tightly closed and attached to the klinostat.

The amount of the centrifugal force may be calculated from the following formula :

$$\frac{4 \pi R \text{ (in meters)}}{gt^2}, \frac{4 \pi^2}{g} = 4.024, \text{ a constant.}$$

$$4.024 \times \frac{R}{t^2} = \text{no. of } g \text{ (gravity).}$$

R = radius expressed in meters.

t = time in seconds of one revolution.

If the centrifugal force is greater than five times gravity it is better to use a modification of the above apparatus, which I have described in a previous note<sup>1</sup> in this JOURNAL, otherwise the intense force will either break the seedling or tear it from its support.

As stated in that note, it is better to insert the seedlings in short pieces of glass tubing and pack them loosely with cotton batting, merely allowing about five millimeters of the root tip to protrude. The pieces of tubing may be attached to the bar with rubber bands. A supply of moisture may be introduced by means of an atomizer.

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## Bacteriology for High Schools.

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### II.

*General Considerations.* The bacteria are so different from any object with which we are familiar, both in size and appearance, and the methods of study so entirely new that some brief general considerations seem necessary before we begin their actual study.

*Nature of Bacteria.* The bacteria have their nearest allies among the plants and are, therefore, to be considered as plants rather than animals.

*Size.* Bacteria are among the smallest of living things. In fact, they are so small that it is very difficult to get even an idea of their actual size. The unit



FIG. 8.—Form types of Bacteria.

of measurement for microscopical objects is a micron (about one twenty-five thousandth part of an inch). If this unit is applied to the bacteria we find that ordinarily their shortest diameter is about one micron. This means that nearly 200 ordinary sized bacteria must be placed side by side to cover a space equal to the thickness of the paper on which this is printed.

*Form.* In form the bacteria are very simple. Briefly, all bacteria may be re-

<sup>1</sup> Journal of Applied Microscopy, Vol. IV., p. 1499.

ferred to three "form types," the sphere, the straight rod, the bent rod. These fundamental form types are shown in Fig. 8. The similarity of form among bacteria, which in reality may be very different, can be imagined when it is understood that into the above mentioned form-types fall at least 1000 fairly well defined bacterial species. The inadequacy of the mere microscopical examination of bacteria will be further realized by the fact that it is absolutely impossible to distinguish, microscopically, between bacteria which are perfectly harmless and those which are most deadly in their action on man. It will, therefore, be quite apparent that in studying the bacteria we cannot rely simply on the microscopical examination of them. For while it is true that we might learn much about the form and structure of bacteria, be able to recognize a number of different species, work out something of their distribution, and perhaps divine something of the relationship which they bear to disease, it is still true that the science of bacteriology dates from what are now known as culture methods.

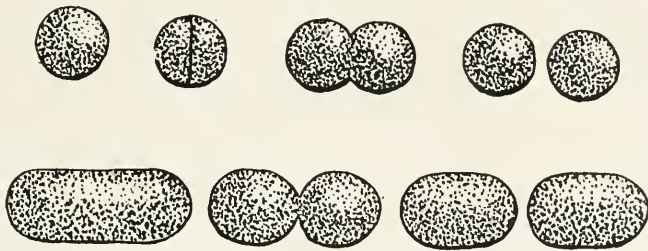


FIG. 9.—Method of Reproduction among Bacteria. The upper series represents the fission of the spherical forms, and the lower that of the rod forms.

By means of cultures we are able to rear the bacteria in large numbers and study, not the single individual, but aggregates of similar bacteria which usually form masses visible to the naked eye, and frequently these masses or "colonies" as they are called are of considerable size.

*The rate of multiplication among Bacteria.* A single generation, which is to be considered as the time elapsing between two successive divisions, is frequently only the fraction of an hour. In some species the individual may grow to maturity and reproduce in twenty minutes. The rate of multiplication thus becomes enormous as time goes on. Some idea of this rate is obtained in the following table :

No. of Generations	No. of Individuals.	Weight.
1	1	0.000,000,000,024,243,672 gr.
24	16,500,000	0.0004 grain-plus.
48	47,000,000,000,000	One pound-plus.
78	—	825 tons.
168	{ 100,000,000,000,000,000 000,000,000,000,000,000 000,000,000,000,000	A mass about the size of the world.

It is evident that such a rate as this cannot long be kept up, but theoretically it is possible and practically it is true that a few germs which form a mass so small that it requires the highest powers of the compound microscope to see them can in a few hours, on a good culture medium, as a cooked potato, become numerous enough to form a mass large enough to be readily seen by the naked eye.

*Method of Multiplication.* The bacteria reproduce themselves by elongating to what may be considered an abnormal length, then form a partition across their shortest diameter, which divides and thus cuts the organism in two. These new formed individuals soon grow to maturity and then in turn divide into two, as shown in Fig. 9.

*Culture Material.* A great many different materials may be used as culture media. Most frequently a beef broth is used, either alone or with the addition of certain solidifying substances as gelatin or agar. Milk is an excellent culture medium, as also are blood serum, egg and cooked potato.

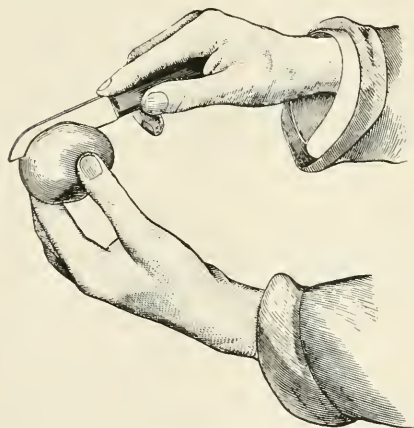


FIG. 10.—Method of Halving Potatoes (after Crookshank).

*Potato Cultures.* The culture medium easiest prepared for the cultivation and isolation of bacteria is the potato medium. The method of preparing and seeding this medium will now be described.

*Cleansing and Sterilization of Potatoes.* Select three potatoes of such size that they will go into the top of a glass tumbler. The skins should be smooth and as free as possible from "eyes" and rotten spots. Wash the potatoes

thoroughly, using a brush to remove the dirt, and with a knife remove the "eyes," injuring the skin as little as possible.

Now place the potatoes in a steamer and cook them about three-quarters of an hour, or until they are done; or the potatoes may be placed in an oven and baked until done. While the potatoes are cooking the receptacles which are to be used may be prepared.

*Preparation of Receptacles.* Six ordinary tea saucers with a like number of glass tumblers are to be washed in boiling water, or better baked in the oven. A circular piece of blotting or filter paper somewhat larger than the top of the tumbler is placed on the saucer and the tumbler inverted over this. If the amount of water which drips from the tumbler is not enough to completely moisten the paper, more should be added.

*Halving the Potatoes.* After the potatoes have been allowed to cool somewhat in the steamer or oven they are to be halved and placed in the receptacles prepared for them. The hands are washed in hot water and wiped with a clean towel. The blade of a potato or case knife is heated very hot in the flame of a Bunsen burner, of an alcohol lamp, or on the surface of a hot stove. A potato



is taken between the thumb and forefinger of the left hand, the knife is held in the right. The potato is halved in the manner shown in Fig. 10. Without allowing the cut surface to come in contact with any object the halves are separated, each half being placed under one of the tumblers. The other potatoes are treated in the same manner. The blade of the knife should be heated before cutting each potato.

If the sterilization has been successful and the subsequent halving and handling of the potatoes done with sufficient care, the halves of the potatoes would show no subsequent change except that due to dessication.

It is important to remember that the covers (tumblers) should not be removed except when absolutely necessary, and then should be returned as quickly as possible.

*Inoculation of the Potatoes.* Remove the cover from one of the potatoes, sprinkle over its surface a very few grains of dirt from an unwashed potato or from the floor. Expose another to the air of the room for half an hour. Over the surface of a third spread with the tip of a knife blade, which has been heated and allowed to cool, a drop of milk or water. Without heating the knife rub the blade over the surface of the fourth potato and again over the fifth half, leaving a space  $\frac{1}{4}$  inch wide next to the edge of the potato which is untouched by the knife. The remaining sixth piece should be left uninoculated as a control or check on the work.

These cultures are now to be placed away from the light and kept at the temperature of the room or slightly above.

*Observation of Cultures.* Examine these cultures daily or oftener and note any changes which occur. After several days describe the color, size, and elevation of areas of growth (or "colonies" as they are called). Any colonies showing bright colors or other marked characters may be transferred to new culture media for further study. In making the transfer a hat-pin heated red hot in a flame, and allowed to cool, can be used. All possible precautions to prevent contamination should be observed.

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A simple and effective method for removing air bubbles from microscopic material is suggested as follows: A small syringe, having a glass barrel, vulcanite mounts, and leather packing to the piston, is the only apparatus required. Select one that is as nearly as possible air tight, unscrew the top and remove the piston. Close the nozzle with a small piece of beeswax, half fill the barrel with distilled water, and into this drop the section or tissues to be treated. Replace the piston and screw on the top. The syringe being inverted and the plug of wax removed, the air is driven out of the barrel by raising the piston till the water begins to flow out of the nozzle, after which close the aperture with the finger and lower the piston. A partial vacuum is thus formed, and the air rapidly escapes from the cells of the tissue, collecting in the point of the syringe. By removing the finger and raising the piston the liberated air is forced out; this may be repeated several times as long as air is being expelled from the material. The same mode of operating is applicable to objects that are to be mounted in Canada balsam if oil of turpentine be used instead of water, and if the objects to be mounted are quite dry before immersion in the turpentine.—*Knowledge*.

## The Technique of Biological Projection and Anesthesia of Animals.

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### XIII. THE ANESTHESIA OF ANIMALS.—Continued.

*Nymph of Dragon-fly.*—These nymphs are usually found in considerable numbers, during fall and spring collecting trips, on submerged wood and plants. They are readily kept alive in batter-jar aquaria, but must be removed from aquaria in which hydras are to be kept, as they eat the latter. The points of special interest which they present to the student include the tracheæ and tracheal gills, the brain and its relation to the eyes, and the heart which displays valvular action with unusual clearness. The heart is in the next to the last somite of the abdomen, between the right and left tracheal trunks. Its largest valves are close to the posterior end, are set in the vertical plane, usually work at a comparatively slow rate, and display valvular action clearly under a low power objective.

As the nymphs differ greatly in color and transparency, the lightest colored specimens should be selected for studies of their anatomy. Place the nymph in a mixture of equal parts of water and one per cent. chloretone solution. If this strength does not soon bring on a state of anesthesia, add more chloretone, or, in case the specimen is very resistant to its action, place it in full strength chloretone solution. The specimen from which the accompanying illustrations were made, lived for about an hour in a small cell filled with the full strength solution.

The accompanying engravings, Figs. 7 and 8, pages 2224 and 2225, taken from photographs of a live nymph as projected on a screen for class study, show the distribution of the more important tracheal trunks which are seen as dark lines in the head, prothorax, terminal segments of the abdomen and tracheal gills. Extending across the head between the eyes is the brain, in which the tracheæ are narrow and dendritic. The eyes, which were easily seen to be compound in the large view on the screen, receive special tracheæ. While tracheal gills are not unusual, this species shows clearly their connection with the main tracheal trunks of the abdomen and their fine terminal branches in the plate-like gills.

*BRANCHIPUS.*—The absence of a carapace renders these animals so transparent that their anatomical structure, the peristalsis of the intestine, pulsation of the heart, and flow of the blood are easily studied in anesthetized specimens. The rate of movement of the phyllopods may be perfectly controlled by the use of chloretone, and, in weak solutions of the drug, the animal's curious normal mode of swimming with the phyllopods up is reversed and the dorsal side assumes its usual position, as seen in most species of animals.

To study the effect of weak solutions on their swimming, place specimens of branchipus in water sixteen to twenty parts with one part one per cent. chloretone. For study under dissecting or compound microscope in the anesthetized state, place them in a watch glass with the same strength of solution named

above, and add one per cent. chloretone solution drop by drop until the desired condition is secured. With this species, as with nearly all others, which are readily brought under the influence of the anesthetic, it is best to return the animals to clear water as soon as their study has been completed, as repeated applications of the drug are less fatal than is a single long continued treatment. For example, branchipus was to be projected before two classes and was treated as follows: first, shown in normal activity; second, without removing it from the projection microscope chloretone was added and the gradual change to the state of anesthesia was observed; third, under higher power objective a study was made of its anatomy and the functional activity of some of its organs; fourth, it was returned to clear water and soon resumed its normal activity, after which the same series of operations was repeated for the second class.

NOTE.—Articles I–VII of this series were devoted to the consideration of available lights for micro-projection, the necessary apparatus and its adjustment; articles VIII–XIV describe the methods of anesthetizing typical animals with chloretone; and the remaining articles will deal with the technique of mounting live plants and animals in the different forms of cells and their manipulation on the projection microscope. Among the hundreds of species which might be used, a few must be selected to illustrate typical methods. The author desires to make the work as generally useful and helpful as possible, and, to this end, would be pleased to receive from those who are using the projection microscope, or are planning to use it, lists of the live objects which they wish to project, and these lists will be utilized in the selection of types to be considered in the articles which are to follow. Also, all who have inquiries in reference to difficulties which they have experienced in carrying out the directions published in the preceding articles are invited to state them in letters to the author and they will be given attention. Address correspondence to No. 5715 Monroe avenue, Chicago, Ill.

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University of Chicago.

## Laboratory Outlines for the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

### THE HIGHER FUNGI AND LICHENS.

XXXI. *Saccharomyces cerevisiæ* Meyen. Beer and Bread Yeast.

Class, Ascomycetes. Order, Saccharomycetales. Family, Saccharomycetaceæ.

To obtain yeast plants in active, vegetative condition, take a piece of ordinary dry yeast cake and put it in a glass of water containing a small amount of sugar. Keep over night in a warm place.

1. Mount some of the water containing yeast plants and study under high power. Draw several of the large oval cells present; also a short, branched filament of cells.

2. Notice the formation of new cells by process of budding. Draw a number of cells showing the several stages in the formation of a daughter cell.

3. Compare the size of a yeast cell with one of the bacteria present.
4. Stain with iodine solution. Notice, the yellowish-brown color of the yeast plants and the blue of the large starch grains of the yeast cake. Is there any starch in the yeast cells?
5. Draw a large cell carefully, showing granules in the protoplasm and one or more large vacuoles.
6. NOTE. Yeast plants produce alcoholic fermentation in saccharine solutions. Dry bread yeast is usually a form of the beer yeast, and is known as "surface yeast."

XXXII. *Morchella esculenta* (L.) Pers. Morel.

Class, Ascomycetes. Order, Helvellales. Family, Helvellaceæ.

This edible morel is common in spring and summer in moist woods and shady hillsides. Specimens may be preserved in 70 per cent. alcohol.

1. Make a careful sketch of the entire, fleshy, fruiting body, representing the stalk and the deep-pitted pileus on whose surface the asci are borne.
2. Tease out a piece of the stalk and mount in water. Examine under high power and draw some of the mycelial threads. Note that the entire body is a spurious tissue of interwoven hyphæ.
3. Tease out a small piece of the pileus, mount, and study the asci. Draw. How many spores in an ascus? How do the asci open at the tips?

XXXIII. *Aspergillus herbariorum* (Wigg.) Fisch. Common Green Mould.

Class, Ascomycetes. Order, Aspergillales. Family, Aspergillaceæ.

This mould is exceedingly common on improperly canned fruit, on cheese, and on decaying plants; especially on plants in press for the herbarium when the driers are not frequently changed. The conidial stage is of a greenish color while the ascospore stage is of a bright yellow-orange to the naked eye.

1. Conidial Stage. Mount carefully in water and study under low power. Under high power draw a conidiophore with conidia. Describe. How are the conidia developed? Draw a piece of the vegetative mycelium, showing the transverse septa.
2. Ascus Stage. Mount some of the white mycelium around the margin of the yellow centre. Under high power draw one of the peculiar coiled hyphal bodies present. These represent the conjugating branches, from which a fruiting body develops.
3. Draw the mature fruiting body (ascocarp) under high power, from a mount of the yellow colored mycelium. Notice the asci containing ascospores.
4. Crush the ascocarps under the cover-glass and draw an ascus with spores. Describe the life history of the plant. The ascocarp may be compared in a general way with the cystocarp of Polysiphonia.

XXXIV. *Uncinula salicis* (DC.) Wint.

Class, Ascomycetes. Order, Perisporiales. Family, Erysibaceæ.

This powdery mildew grows as a parasite on the leaves of various species of willow and can usually be found without difficulty in the autumn. It forms a



white layer on the surface of the leaf in which minute black bodies are situated. It may be preserved in 70 per cent. alcohol or kept in a paper box.

1. Moisten a leaf with water and scrape off some of the mycelium containing the black bodies (cleistothecia). Mount in water and examine under low power. Under high power draw a cleistothecium with appendages.

2. Draw a small piece of the mycelium showing the transverse walls in the hyphæ.

3. Crush some of the cleistothecia under the cover-glass by pressing and rubbing carefully over the surface with the handle of the needle. Draw an ascus containing ascospores. How many asci in a cleistothecium? How many spores in an ascus?

XXXV. *Ustilago maydis* (DC.) Tul. Corn Smut.

Class, Hemibasidii. Order, Ustilaginales. Family, Ustilaginaceæ.

The corn smut may be collected in summer and autumn and kept in a dry condition in paper boxes.

1. Make a naked eye sketch of one of the large, black, smut nodules. On what parts do the smut nodules develop?

2. Mount some of the black powder and study under high power. Draw a number of the small spores. These are usually known as chlamydospores or teleutospores. Describe the color, surface and shape.

3. Make a hanging-drop culture of the spores with dilute, boiled, stable-manure water. Smut spores germinate quite readily, but it is best to let them freeze before making the culture. Watch the germination from day to day and note the formation of the small promycelium or basidium which develops a number of hyaline basidiospores. Note that the smut plant is a parasite while the promycelium is a saprophyte.

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## Syllabus of Work in Biology for High Schools.

The following is the syllabus in biology, including botany, zoölogy, and physiology, for high schools, as adopted by the Board of Superintendents, Department of Education, city of New York. We believe it will be found exceedingly valuable to science teachers as a suggestion in outlining their courses, and therefore publish the syllabus in full:

### GENERAL RECOMMENDATIONS.

As a more comprehensive view of each of the subjects embraced under biology (botany, zoölogy, and physiology) can be obtained by the consideration of a large number of topics, it is suggested that many topics be studied somewhat briefly, though it is important that a few typical specimens should be studied intensively. The conditions for favorable study of some of the topics outlined under botany and zoölogy vary among the different high schools of the city, and vary for the same school at different seasons, and on this account considerable latitude may be exercised in the choice of topics. The order of presentation of the topics under each subject, and the order in which the three subjects should be taught is of less importance than the presentation of them under the most favorable conditions obtainable.

In the study of botany and zoölogy three lines of work should be carried on.

First—Field work, including,

1. Work conducted by the teacher outside the school building in the woods, fields, and parks.
2. Work carried on by the individual pupils and reported to the teacher for suggestions and assistance.

Second—Laboratory work, including,

1. The study of living plants and animals in the school room.
2. The study of prepared specimens, dissections, and microscopic slides.
3. The careful dissection of a few important types.
4. Careful drawing and descriptions of specimens should be made.

Third—Recitation work, including,

1. A consideration of the facts observed in the field and laboratory.
2. A consideration of important plants and animals related to the types already studied.
3. A consideration of the elementary facts and principles relating to the classification of plants and animals.
4. Written descriptions of some of the specimens studied.

The five periods per week given to biology should be divided as nearly equally as possible between laboratory and recitation work.

#### *FIRST YEAR—First Half (Term I).*

##### BOTANY.

Required topics : I, II, III, IV, VII.

Optional topics : V, VI.

For first year pupils, the study of botany should include such simple chemical and physical experiments as are necessary for the understanding of plant life.

#### I. SEEDS AND SEEDLINGS.

Topics : Monocotyledons, dicotyledons, gymnosperms; kinds of food stored, determined by experimental tests; conditions of germination; the development of the elements in the embryo into organs of the seedling.

#### II. ROOT.

Topics : General structural characteristics; secondary roots, root hairs, root cap, and growing point; raw food material, osmosis, movements of sap, storage of food; arrangement of tissues.

#### III. SHOOT.

##### 1. Stem.

Topics : External features; arrangement of branches; modifications in form to fit environment; underground stems; tissue arrangement in monocotyledons and dicotyledons; functions of stems.

##### 2. Bud.

Topics : Relation of buds to the formation of branches; kinds of buds; bud protection; vernalization.

##### 3. Leaf.

Topics : Structure; comparison of monocotyledons and dicotyledons in leaf and stem; leaf arrangement and light relations; transpiration; raw food material, photosynthesis, digestion, assimilation; respiration; fall of leaf.

#### IV. FLOWER AND FRUIT.

##### 1. Flower.

Topics : Structure and function of parts of the flower ; adaptations for pollination ; fertilization ; principal forms of inflorescence.

##### 2. Fruit.

Topics : Relation of flower to fruit and seed ; typical fruits ; dispersal of seeds.

#### V. TAXONOMY.

Topics : Familiar types representing at least ten of the large families of flowering plants should be selected, and the characteristic features observed, unessential details being passed over. Such work incidentally involves the use of the manual. It should be understood, however, that the object of this work is not to develop complete knowledge of the structures of these groups, but rather to enable the student to recognize important plant families in the laboratory and in the field.

#### VI. CRYPTOGRAMS.

Topics : Suggested types will be found in the list of material. The name of each type will itself suggest the method of study to be followed.

#### VII. ECOLOGY.

Topics : Reference to preceding topics will make it clear that the relation of plants to their environment may be made a subject of frequent and profitable study. All the facts learned through the course should be brought together at the close and given their broadest significance, to the end that the student may be trained in the practice of interpreting facts, and that he may recognize the inherent unity of the science of botany.

### SUGGESTED LIST OF MATERIAL IN BOTANY.

#### I. SEEDS AND SEEDLINGS.

Bean, pea, morning-glory, castor-bean, pumpkin, barley, sunflower, corn, pine, peanut, Windsor bean.

#### II. ROOT.

Cherry, apple, turnip, carrot, parsnip, corn, ivy, spiderwort, oat, radish, barley, sunflower, duckweed, water-hyacinth, willow.

#### III. SHOOT.

##### 1. Stem.

Horse chestnut, beech, hickory, magnolia, apple, locust, osage-orange, cat-brier, corn, ivy, grape, woodbine, vetch, hop, iris, sedge, potato, hyacinth, onion, gladiolus.

##### 2. Bud.

Horse-chestnut, beech, apple, cherry, hickory, magnolia, geranium, ailanthus, boxelder, butternut, sumach, sassafras, bryophyllum.

##### 3. Leaf.

Horse-chestnut, hickory, beech, apple, rose, elm, laurel, maple, locust, pea, mullein, dandelion, grass, corn, Solomon's seal, thistle, pine, clover, nasturtium, hydrangea, India-rubber plant, house-leek, loosestrife, bed-straw, pitcher plant, sundew, cactus, Venus' fly-trap.

#### IV. FLOWER AND FRUIT.

##### 1. Flower.

Trillium, buttercup, sweet-pea, apple, cherry, Weigela, hepatica, jack-in-the-pulpit, anemone, columbine, narcissus, adder's-tongue, cinque-foil,

strawberry, violet, wild pink, azalea, arbutus, cranesbill, marsh marigold, blackberry, malva rotundifolia, pine, mountain laurel, white daisy, jewel weed, butter and eggs, ox-eye daisy, bachelor's button, evening primrose, mint, golden-rod, chrysanthemum, gentian, syringa, bouvardia, lupine.

## 2. Fruit.

Maple, elm, linden, pine, cockle-burr, beggar's-ticks, stick-tights, dock, chestnuts, locust, pea, bean, peanuts, sensitive partridge pea, dandelion, milkweed, ailanthus, acorn, oats, wheat, monkshood, poppy, lotus, witch-hazel, garden balsam, fleshy fruit.

## V. TAXONOMY.

## VI. CRYPTOGAMS.

Pleurococcus, Hæmatococcus, Sphærella; yeast; Bacteria; Spirogyra; Zygnema; Mucor, Rhizopus; Fucus; edible fungi and fungi injurious to vegetation: moss, fern, Equisetum.

## *Second Half (Term II).*

## ZOOLOGY.

Required topics: I, II, III, IV, V, VI.

Optional topics: VII, VIII, IX, X, XI, XII.

For first year pupils the study of zoölogy and physiology should include such simple chemical and physical experiments as are necessary for the understanding of animal life.

## I. ARTHROPODA.

### 1. Crustaceans.

Topics: External and internal morphology; high specialization in appendages; inherent similarity of parts of appendages; development and metamorphosis; protective coloration; economic interests.

### 2. Insects.

Topics: External morphology; habits of living; metamorphosis; cross pollination of flowers; protective coloration; economic entomology; principles of classification in zoölogy, illustrated by a brief comparison of insect orders; distribution.

### 3. Spiders.

Topics: Structural characteristics; web-building; capturing of prey; care of young.

## II. AMPHIBIANS.

Topics: External and internal morphology; development and metamorphosis; protective coloration; habits of living in larval and adult form; distribution.

## III. COELENTERATA.

Topics: Radial symmetry; alternation of generations (in Hydrozoa); formation of coral (in Actinozoa); simple digestive cavity; budding; protective cells; adaptations to sessile life.

## IV. PROTOZOA.

Topics: Structure and activity of protoplasm; ingestion; digestion, assimilation, excretion, respiration, locomotion, reproduction; the cell as a structural and a physiological unit.

## V. BIRDS.

Topics: External and internal anatomy; seasonal coloring; migrations; nest-building; song; care of young; economic relations.



VI. MAMMALS.

Topics : Structure ; specialization in structure and in habits ; instinct and reason ; care of young ; geological development.

VII. MOLLUSCA.

Topics : General arrangement of organs ; special activities ; adaptation to surroundings ; economic importance.

VIII. FISHES.

Topics : Arrangement of internal organs ; exoskeleton and endoskeleton ; adjustment between structure and function ; distribution ; fishery industry.

IX. ANNULATA.

Topics : Bilateral symmetry ; segmentation ; general anatomy ; activities ; regeneration ; parasitism in allied forms ; economic interests.

X. ECHINODERMATA.

Topics : Structure ; development and metamorphosis ; differentiation into tissues (ectoderm and endoderm) ; habits of obtaining food ; locomotion ; adaptations for protection.

XI. PORIFERA.

Topics : Beginning of differentiation in cell structure ; sessile habit ; incurrent and excurrent openings ; skeleton ; sexual reproductive cells ; economic interests.

XII. REPTILES.

Topics : Morphology : exoskeleton and endoskeleton ; relationship to other vertebrates ; harmless and injurious forms ; habits ; distribution.

NOTE.—The material of the appropriate groups should be used for illustrative purposes in comparative study in connection with human physiology.

SUGGESTED LIST OF MATERIAL IN ZOOLOGY.

I. ARTHROPODA.

(1) Crustaceans.

Crayfish, lobster.

(2) Insects.

Grasshopper, cricket, cockroach, dragon fly, caddis-fly, cicada, plant-louse, squash bug, house-fly, mosquito, butterfly, moth, beetle, bee, ant, gall-fly.

(3) Spiders.

Garden spider, house spider.

II. AMPHIBIA.

Frog, toad, salamander.

III. COELENTERATA.

Sea-anemone, Hydra, hydroid, medusa, Scyphozoa.

IV. PROTOZOA.

Paramœcium, Vorticella, Stentor, Stylonichia, Didinium.  
Euglena, Amœba.

V. BIRDS.

Any bird.

VI. MAMMALS.

Any mammal.

VII. MOLLUSCA.

Clam, mussel, snail, slug, squid.

## VIII. FISHES.

Any bony fish.

## IX. ANNULATA.

Earthworm, sandworm (Nereis).

## X. ECHINODERMATA.

Starfish and Sea-urchin.

## XI. PORIFERA.

Grantia, Spongilla, commercial sponges.

## XII. REPTILES.

Turtle, snake, lizard.

## FOURTH YEAR (Elective).

The syllabus of the elective work in biology for this year of the curriculum shall correspond to the requirements outlined by the College Entrance Examination Board.

## PHYSIOLOGY.

*Second Half (Term II).*

This subject is to be studied during the term as part of the work in Zoölogy.

- I. Introductory: Man's place among the animals; general composition of the body; general survey of organs and tissues.
- II. Study of foods and nutrition as the source of physiological energy.
- III. Study of the structure, functions, and hygiene of the skeleton, the muscles, the digestive organs, the circulatory organs, with the composition of blood and lymph; the respiratory organs: the kidneys and skin; the nervous system and special senses. Effects upon these of alcohol and narcotics.

## LABORATORY PHOTOGRAPHY.

L. B. ELLIOTT.

Devoted to Methods and Apparatus for Converting an Object into an Illustration.

## A Simple Method of Copying for the Making of Lantern Slides.

For the past two years I have constantly had occasion to make lantern slides from cuts in books. Those who have had experience in this line know well that simple as such work may seem in theory, in practice it is troublesome to carry out. The books containing the cuts are usually heavy and their binding stiff. It is often impossible to stand them up to copy from, and the various copying stands that have been devised are large, clumsy, and not easily portable. The book from which a cut is required may be in a friend's house, or in a library, and cannot be borrowed. Again, when many negatives have to be made, centering of the subject, focussing, and change of holders, etc., take up much time, and the request recently made me by my colleague in the chair of physiology for a hundred slides from four different books means more than a morning's work. In the production of such slides I have tried all the approved methods; used a

special copying stand in which the book is pressed flat against a plate glass surface by blocks and screws from behind; have employed expensive lenses from a sixteen-inch anastigmat downwards; have tried all kinds of plates (with a preference for a process plate or lantern slide); and yet with all these advantages I have not succeeded in getting any better results than are obtainable by the simple procedure I am about to describe.

Most cuts in books are about the right size for making lantern slides, so that in a general way they require to be copied at the same size, or on a slightly reduced scale. Whilst reading a recent work on photographic optics I was much impressed with the great depth of focus obtainable by stopping down a short focus lens, and the idea flashed into my mind that it could be utilized in using a small fixed focus hand camera for making my negatives from books; and I proceeded at once to test it. Amongst quite a collection of cameras I possess an Eastman Bullseye kodak, a small affair carrying a  $3\frac{1}{2} \times 3\frac{1}{2}$  film, and armed with a five-inch fixed focus lens. I ascertained by means of a set of opti-



FIG. 1.—A carcinomatous stomach photographed direct from specimen (under water) with kodak and supplemental + 3 lens at 15 inches.

cian's test glasses what strength of supplemental spectacle lens was required to bring a cut in a book on to the ground glass full size, and found I could do it with a  $5\frac{1}{2}$ -inch (7 diopter) lens, and that I could obtain the image half the size with a 13-inch (3) lens. The distance of object from lens was in the first case  $7\frac{1}{2}$  inches, in the second 12 inches. I could have worked this out by formula, but it is easier for me to test than calculate. I now knew that I could photograph an object half natural size at 12 inches distance therefrom if I put a 13-inch or 3 diopter spectacle lens in front of my kodak lens. I had further learned from the book I had read, that if the lens were stopped down to F-45 or F-60 I should have a focal depth of about six inches; in other words, I could put my camera nine inches from the cut to be copied, or fifteen inches, and get a sharp picture in either case. This I proceeded to test. I put a book open on the table, and above it on the ring of a retort stand I placed my camera with its supplemental lens and small stop, and I proceeded to make a series of copies at

distances of from eight inches to sixteen inches from the camera, raising the ring of the retort stand one inch each time. I then developed the film and found

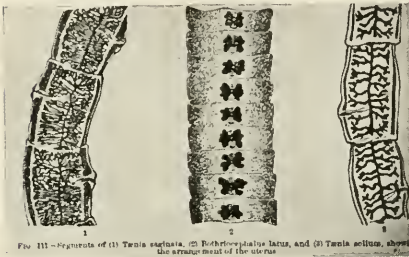


FIG. 2.—Copied  $\frac{1}{2}$  size from book with + 3 supplemental spectacle lens and Bullseye kodak.

film or thereabouts, attach a supplemental spectacle lens mounted in a cap to fit over the original lens. Ascertain by trial the distance at which the combination gives a sharp focus with a large stop. In the case of a Bullseye kodak or any other camera carrying a five-inch lens, this is a 13-inch spectacle lens—and



FIG. 3.—*Fasciola hepatica*, natural size, viewed from ventral surface. Copied full size from book with Bullseye kodak and + 7 spectacle lens at  $4\frac{3}{4}$  inches.

the correct distance is 12 inches for a half reduction, or a  $5\frac{1}{2}$ -inch lens at  $4\frac{3}{4}$  inches for same size. For use put on the supplemental lens, stop down to smallest diaphragm,\* and place camera over book at any distance between 10 and 14 inches. The book should be flat on the table and the camera can be maintained in position by a pile of books on either side, or, as I prefer, by a simple little stand I have devised therefor. Exposure is best effected by burning eight inches of magnesium ribbon above the book by the side of the camera. Diffused daylight in a room requires about two min-

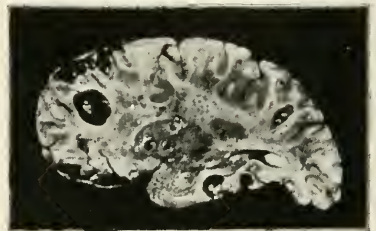


FIG. 4.—*Cysticercus cellulosæ* in the human brain. Copied  $\frac{1}{2}$ -size from book with Bullseye kodak and + 3 spectacle lens.

utes. The advantages of this method of working lie in the extreme simplicity of the apparatus; its portability enabling copies to be made in libraries, etc.; the ease with which a book lying open is kept flat; the elimination of focussing, with its loss of time; and the rapidity with which films may be changed as compared with plates. Its disadvantages I have yet to discover.

I might mention that this great depth of focus makes it equally valuable in making negatives of small solid objects, beetles, geological specimens, etc.

College of Physicians and Surgeons, San Francisco.

H. D'ARCY POWER.

\* The smallest stop on a kodak is no longer small when the focus is reduced by the addition of a supplemental lens. This is easily corrected by placing a piece of cardboard in front of the lens with the necessary perforation; in the case of the full sized reproduction, about  $\frac{1}{15}$ -inch.



## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Nemec, B. Ueber centrosomähnliche Gebilde in Vegetativen Zellen der Gefäßpflanzen. Ber. d. deutsch. bot. Gesell. 19: 301-310, pl. 15, 1901.

fixing fluids were: Flemming's chromo-osmo-acetic acid; chomo-acetic acid; and a mixture of picric, acetic and sulphuric acids. The most successful stains were: Fuchsin-S with or without Haidenhain's iron alum hæmatoxylin; Flemming's safranin-gentian violet-orange; and Smaragdgrün with fuchsin-S.

It was not difficult to find bodies resembling centrosomes and which would doubtless be interpreted as such by those who expect to find centrosomes in every cell. The writer, however, comes to the conclusion that there are no genuine centrosomes in the vegetative cells of the vascular plants and none in the reproductive cells, unless blepharoplasts are centrosomes, and he believes that they are not. The figures look like those which are familiar to anyone who has made preparations of mitotic figures in root tips. Prof. Némec states that with the same methods he was able to differentiate clearly the centrosomes of the liverworts.

C. J. C.

Gerassimow, J. J. Ueber den Einfluss des Kerns auf das Wachstum der Zelle. Bull. de la Soc. Imp. des Natural. de Moscou. Pp. 185-220, 47 tables and 2 plates, 1901.

In this paper Prof. Gerassimow records the results of his extensive experiments upon the influence of the nucleus upon the growth of the cell. *Spirogyra* was the plant used and the conclusions depend upon a comparison of the behavior of nucleated and non-nucleated cells. Previous investigators have worked with enucleated portions of cells, but the present observations were made upon genuine non-nucleate cells. To secure such non-nucleate cells, material with dividing nuclei was selected and the mitotic division was disturbed so as to move the dividing nucleus from its central position toward one side; on the completion of the partition, one of the cells—in successful cases—would be left without a nucleus, although its chomatophores and other structures seem to be normal. Non-nucleate material can be secured by lowering the temperature to somewhere near the freezing point for thirty minutes or an hour, the water, however, not being allowed to freeze. As is well known, *Spirogyra* usually divides late in the evening or at night, but can be delayed until morning by keeping the temperature sufficiently low, division taking place when the temperature is allowed to rise. The writer preferred to use material found dividing spontaneously. Such material was placed in a suitable vessel, surrounded with snow or crushed ice for about an hour, and was then brought gradually to the room temperature. On the following morning many non-nucleate cells and incomplete chambers

For this work Prof. Némec used root tips of *Blechnum brasiliense*, *Diplazium pubescens*, *Dracena arborea*, *Hibiscus calycinus* and *Alnus glutinosa*. The

In this paper Prof. Gerassimow records the results of his extensive experiments upon the influence of the nucleus upon the growth of the cell. *Spirogyra* was

would be found. Although division may be induced by ether, this method was very little used.

The following are some of the conclusions :

The growth of a cell which has a superabundance of nuclear material is more vigorous than that of the ordinary uni-nucleate cell. The cell wall, the chromatophores and, apparently, the protoplasm also grow more vigorously. Such cells divide only after they have reached a noticeably larger size. Non-nucleate cells can grow somewhat in length. The non-nucleate chamber (which is distinguished from the non-nucleate cell by a larger or smaller opening in the partition separating it from its sister cell with the superabundance of nuclear material) grows more vigorously than the non-nucleate cell. Cells with a superabundance of nuclear material can conjugate with each other or with ordinary cells and the size of the zygospore is in direct relation to the size of the conjugating cells.

C. J. C.

Reed, H. S. The Development of the Macrosporangium of *Yucca filamentosa*. Bot. Gazette, 35: 209-214, 5 figs., 1903.

The archesporial cell gives rise to a tapetal cell and four megaspores, the latter usually in a row, although occa-

sionally the two nearest the micropyle lie side by side. There is an abundant secretion from cells of the funiculus. The secretion is regarded as a substance not nutritive in itself, but as a medium through which the substance capable of attracting pollen tubes diffuses outward toward the micropyle.

Flemming's weaker solution and Worcester's killing fluid were used for fixing. Since the latter solution is not familiar to most botanists, the formula may be of interest :

Mercuric chloride, saturated aqueous solution	96 parts
Formalin (40 per cent. formaldehyde)	- 4 parts
Acetic acid, 10 per cent.	- - - 10 parts
Formic acid to each liter solution	- - - 5 drops

Material should be washed in 70 per cent. alcohol.

It is evident that this solution would penetrate and fix even more rapidly than the usual mercuric chloride solutions. It seems to be worth a test.

C. J. C.

Cavers, F. On Saprophytism and Mycorrhiza in Hepaticæ. New Phytologist, 2: 30-35, 1903.

Fungi inhabiting Hepaticæ are divided into two classes; viz., those that

attack the sporogonium and those that live exclusively in the tissues of the gametophyte. A careful investigation of the life history of the fungi in several species of liverworts showed that the infected sporogonia, as a rule, remain imperfectly developed, showing a few irregular divisions and remaining abortive, while the capsule is filled with fungus hyphæ and spores. The conclusion is that in such cases the relation is one of pure parasitism.

In *Conocephalus (Fegatella) conicus* and some others, the presence of the fungus in the tissues produced a more luxuriant liverwort, appearing to form a mycorrhiza, causing the liverwort to become largely saprophytic. The conclusion is that in many cases the relation of fungus and liverwort is one of symbiosis. It is suggested that the presence of sphagnol in the tissues of *Conocephalus* and others serves to regulate the growth of fungus and prevents symbiosis from passing over into saprophytism.

J. F. GARBER.

Chicago.

# CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

**Shaw, Philip E.** Electric Method of Taking Microscopic Measurements. *Journ. Roy. Micr. Soc.*, Pt. 6, 625-630, 1902.

A small apparatus has been made by modifying designs described two years previously, called "The Simple Electric Micrometer," capable of giving measurements of  $\frac{1}{1000}$  of mm. or less. This has two points in its favor—first, it is electric; second, it is a direct method.

In the diagram (Fig. 1) the essential parts are shown. The slide, *sl*, is mounted on the stage, *s*, *o* being the microscope objective. A screw, *sc*, is brought up to nearly touch the edge of the stage. This screw is carried by a nut, *n*, and has a graduated disc, *d*, with a milled head, *m*, attached to it. If

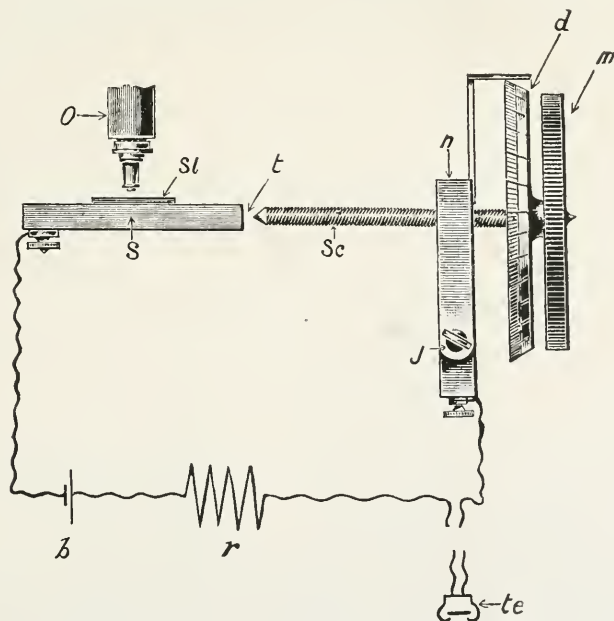


FIG. 1.

the screw should have two threads to a mm., and the graduated disc 500 divisions, then a movement of the screw-disc by one division corresponds to a movement of the screw-point, *t*, by .001 of a mm. or one micron. The micrometer-screw is supported on a stand quite separate from the microscope, so that it can be packed separately. There is a universal joint, *j*, between the screw and the stand, so that the screw can be raised, lowered or pointed in any desired position and then clamped. Suppose the screw and stage to be brought into

contact, an electric circuit is completed through a battery,  $b$ , a resistance,  $r$ , a telephone receiver,  $te$ , a nut,  $n$ , and thence through the screw,  $sc$ , and stage,  $s$ , to the battery. At each make or break of the circuit at the point,  $t$ , the telephone speaks. To use this apparatus, move the stage by rack and pinion, or

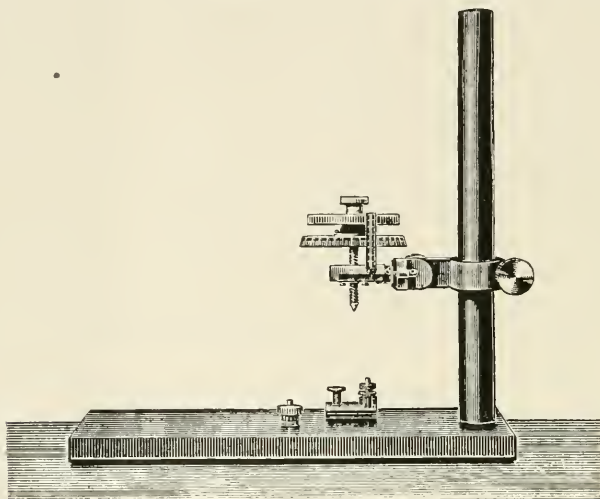


FIG. 2.

other adjustment, till the desired object is on the cross wire, bring the screw,  $sc$ , into contact with the stage,  $s$ , when the telephone speaks observe the reading,  $R_1$ , of the disc,  $d$ . Get the other side of the object coincident with the cross wire, bring the screw into contact again and get a new reading,  $R$ , of disc.

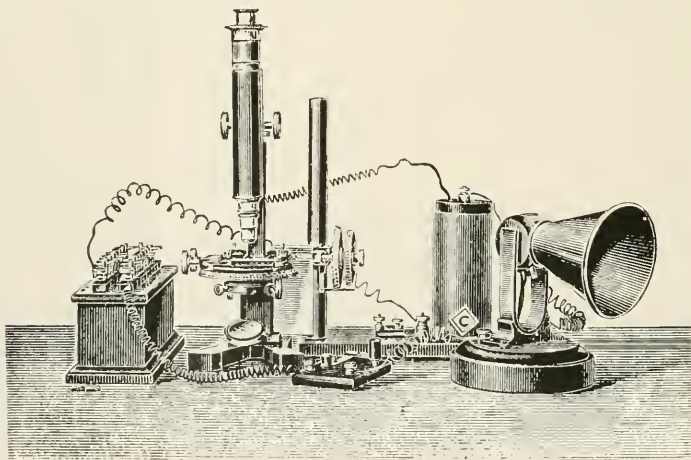


FIG. 3.

Diameter of the object will be  $R_1 - R$ . Fig. 2 shows the micrometer alone, and Fig. 3 the whole apparatus for microscopic measurement. This method has been tested exhaustively for two years and is quite reliable if two conditions are observed. (1) Both the contact surfaces of the screw and the stage must be



metallically clean: fine emery cloth should be used on them until they are free from lacquer, oil, etc. (2) Vibrations should be avoided. Mount the apparatus on a steady table and handle the screw delicately in taking measurements. Any cell will do for the circuit and any telephone receiver will act, though a "loud speaker" is most convenient. For resistance a few hundred ohms serves, its object being to keep the current small, but it is not an essential. Any spare screw on the microscope serves for completing the circuit or simple lashing to an unlacquered surface works.

This electric micrometer has the advantage of being a direct method, thus giving surer results than eyepiece micrometers. It is more enduring than the stage with a graduated screw movement, since the work of carrying the stage does not rest on the micrometer screw, causing wear; a larger disc can be used without inconvenience, and the apparatus put away when not in use. No difficulty has been experienced in reading to  $\frac{1}{4}$  micron, and with the insertion of a capstan-pin into the edge of the milled head of the screw, a more delicate angular movement may be given to the screw, and readings of  $\frac{1}{10}$  micron obtained.

A. M. C.

**Blackman, M. W.** Methods in the Preparation of Material for Study of Spermatozoa of Myriopods. Kansas University Quarterly, 10: 2, 1902.

The material was obtained from a species of *Scolopendra* which is found abundantly in the Southwest. In the manipulation of the material two fixing reagents were used: Flemming's chromosmium-acetic mixture and Gilson's nitro-acetic-sublimate mixture. Both of these mixtures gave excellent results, but Gilson's fluid was the better. The fixation with this was perfect, there being no shrinkage or other apparent distortions. The only disadvantage of the Gilson fixative is the difficulty experienced in the later manipulation. When embedded in paraffin the material is so soft and spongy that it folds upon the knife in cutting. This difficulty was obviated in the following manner: The material was gradually carried up to absolute alcohol, from which it was transferred to celloidin and allowed to infiltrate thoroughly. Then the celloidin was allowed to evaporate gradually, until it was of the consistency of thick cream. Finally all the surplus celloidin was removed, and the mass cleared for several hours in chloroform. This accomplished, the specimen was infiltrated with paraffin and embedded in the same substance. The material cut perfectly, without any wrinkling or distortion of the sections, and without any of that shrinkage of the cells which often occurs when the ordinary paraffin method is employed. In staining, a considerable number of reagents were employed. The best results were obtained with Heidenhain's iron-hæmatoxylin, used either alone or in connection with Congo red; Keruschwarz; and the Flemming three-color stain. Fair results were obtained also with Bismarck brown, methyl-green, cyanin, and Auerbach's methyl-green, and acid fuchsin.

C. W. J.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

**Conklin, E. G.** Karyokinesis and Cytokinesis in the Maturation, Fertilization and Cleavage of *Crepidula* and other Gasteropoda. *Journ. Acad. Nat. Sci. Phila.* **12**: 1-124, 6 pls., 1902.

The eggs of *Crepidula* are most favorable for the study of cleavage. They are enclosed in membranous capsules which reagents readily penetrate. Eight to ten thousand eggs may be secured from a single female, 175 eggs being found in a single capsule of *C. plana*. The eggs are small (.136 mm.), of uniform size, and contain much yolk, which stains heavily with most stains. For the study of entire eggs the author teased the living eggs from the capsules into Kleinenberg's picro-sulphuric mixture or Boveri's picro-acetic, where they were left for thirty minutes to two hours. They are then washed in alcohol until they become white and are stained for five to ten minutes in the following solution: Delafield's hæmatoxylin 10 cm.<sup>3</sup>, distilled water 40 cm.<sup>3</sup>, Kleinenberg's picro-sulphuric fluid 10 drops. Eggs so stained are washed in alcohol, dehydrated and mounted in balsam. Eggs of *C. plana* mounted under a thin cover supported at one side by a bit of glass .15 mm. thick can be studied with an oil immersion lens and the relative positions of the nuclear structures determined, as would be impossible in sections. This stain leaves the yolk a transparent yellow, colors the protoplasm a rosy tint, while chromatin, centrosomes, and mid-bodies are blue or black. The method is recommended for eggs of mollusks, annelids and arthropods where there is much yolk. Eggs fixed in Kleinenberg's fluid are more transparent than those in Boveri's picro-acetic, but the cytoplasm is better preserved by the latter. For sectioning the following fixing fluids gave good results: Flemming's, Hermann's, Boveri's picro-acetic, Graf's picro-formol, while a long list of well known fluids gave inferior results. The best results were secured by fixing in Boveri's picro-acetic and staining in Delafield's hæmatoxylin and orange G. Iron hæmatoxylin and Bordeaux red also gave good results, though obscuring centrosomes and asters in the yolk, which stains heavily and resists de-staining. Hermann's safranin-gentian-iodine, Biondi-Heidenhain's mixture, Auerbach's acid fuchsin and methyl-green and other stains were also used. Sections were wont to crumble on account of yolk. This was obviated by double embedding in celloidin and paraffin by Kultschitzky's method, though sections of such material are much folded and are with difficulty flattened.

C. A. K.

**Harmer, S. F.** On the Morphology of the Cheilostomata. *Quart. Journ. Mic. Sci. N.* **46**: 263-350, pls. 15-18, 1902.

Alcoholic material was used for this investigation, and the growing edges of healthy colonies were selected as yielding most satisfactory results. The material was stained without decalcification in dilute borax carmine for 5 to 7 days, and dehydrated in absolute alcohol to which picric acid had been added, and then mounted whole in balsam. Speci-

mens were transferred directly from absolute alcohol to a solution of dried Canada balsam in absolute alcohol. The milky cloudiness produced in preparing this solution and also on placing objects in it disappears when the solution or objects are kept at 60°C. for a few days. This method of mounting is strongly recommended for Polyzoa, which are often badly disturbed by clove oil in clearing. Densely calcified species were studied by removing the basal wall with a scalpel from stained but not decalcified colonies embedded in paraffin. The author is convinced of the great importance of studying the Cheilostomata in undecalcified Canada balsam preparation.

C. A. K.

**Argutinsky, P.** Malariastudien. Zweite Mitteilung: Zur Morphologie des Tertianparasiten (*Plasmodium vivax* Gr. er Fel.). Arch. f. Mik. Anat. u. Entwickl. 61: 331-348, Taf. xviii, 1902.

The author states that the usual method of drying blood preparations results in a distortion of the finer structure of the malarial parasite. Alcohol and

alcohol-ether are likewise poor fixers for nuclear structures. Fixation of dried blood films in aqueous or alcoholic sublimate affords fine elective staining, but the blood corpuscles and their parasites are still subject to the distortion caused by drying. On the other hand, the use of fluid fixers for undried blood is fraught with difficulty, on account of the great elasticity of the erythrocytes, and also because the fresh corpuscles are subject to swelling and shrinkage when placed in the anisotonic solutions used as fixers. Furthermore, the fluid usually washes away a considerable part of the blood smear even when greatest care is used.

To obviate all these difficulties and at the same time secure perfect fixation and fair stainability the author experimented with vapor methods. Formalin fixes well, but interferes with the elective eosin-soda blue staining. Osmic acid was finally selected as the most available fixing agent and was employed as follows: From 6 to 10 drops of a mixture of osmic and acetic acids is placed in a small low watch-glass in a Petri dish. The acids are thoroughly mixed immediately before using in the proportion of 2 cm.<sup>3</sup> of 4 per cent. aqueous solution of osmic acid to one drop of 50 per cent. acetic acid. The slides with blood smears prepared by the method of Janczo and Rosenberger are placed smear down over the watch-glass as quickly as possible after preparation and the Petri dish is closed. The slide is removed after an exposure of thirty seconds, and is allowed to dry at room temperature for a few seconds only. By this method 20 or more preparations may be made in a half hour. The slides are bleached for 5 to 10 minutes in an officinal solution of hydrogen peroxide, washed for 15 minutes in distilled water, dried between filters and then stained. Since the osmium interferes somewhat with the staining it is necessary to use well ripened and strong solutions of the soda-methylen-blue stain. The author recommends 1 per cent. solutions ripened for three months in diffuse daylight. After exposure to the stain for 30 minutes to an hour the preparations are differentiated. Material thus prepared is most excellent for exhibiting the form and nuclear structure of the parasite, even for the youngest stages, and is thus of greatest clinical as well as morphological value.

C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory,  
University of Michigan, Ann Arbor, Mich.

**Porter, W. T.** New Inductorium, Kymograph, Heart Lever, Heavy Muscle Lever, and Square Rheochord. *Proc. Amer. Physiol. Soc. Fifteenth Ann. Meeting. Amer. Jour. Physiol.* 8: pp. xxxv-xli, 1903.

Dr. Porter describes in this paper improved forms of several of the important pieces of physiological apparatus which he has designed. An improved

inductorium is shown in Fig. 1. The primary coil is fastened to the hard rubber head piece, which bears on its outer face the automatic interrupter and binding posts. All the connections of the primary coil and interrupter are made on the face of the head piece in plain view, there being no concealed wires. The secondary coil slides on brass rods fastened to the head piece. Each end of the secondary wire is fastened to a brass bar screwed to the ends of the hard spool. The brass bars bear a trunnion which revolves in a split brass block, the fric-

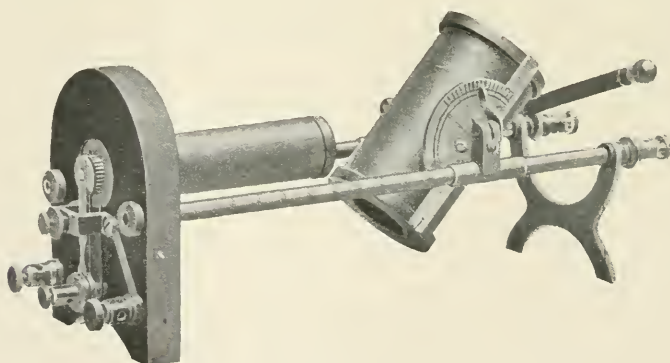


FIG. 1.

tion of which is regulated by a screw. The trunnion block is cast in one piece with a tube 3 cm. in length, which slides upon the slide rods. The secondary spool revolves between the side rods in a vertical plane. When the secondary coil has revolved through 90°, a pin upon the side bar of the secondary coil strikes against the trunnion block and prevents further movement in that direction. The right hand side bar bears a half circle graduated upon one side from 0° to 90°. An index pointer is fastened upon the trunnion block. One side rod is graduated in centimeters. This arrangement for the movement of the secondary coil makes the inductorium as a whole very compact. The side rods end in the secondary binding posts, so that moving the secondary coil does not drag the electrodes. Next the binding posts is placed a substantial "knife switch" short circuiting key, with hard rubber handle.

The most noteworthy single feature about the improved kymograph is the drum. The drum is made of aluminium, cast in one piece, turned true in the



lathe to a circumference of 50 cms. The weight is 600 grams. The drum is borne on a brass sleeve which revolves about a steel shaft. The sleeve and drum may be disconnected from the clockwork and then may be "spun" by hand.

The improved rheochord is in form of a square, as shown in Fig. 2. Its base is a block of hard maple, 12.5 cms. square, upon which is placed a centimeter scale beginning at the 0 post shown on the left side of the figure and ending in the 1-meter post visible in the background to the left. Along the scale, between these two posts, is stretched the first meter of a continuous German silver wire, .26 mm. in diameter and 20 meters long. The remaining 19 meters of this wire are coiled upon a spool, and the free end is fastened to the 20-meter post shown in the background to the right of Fig. 2. The resistance in the 20 meters of German silver wire is so great (about 184 ohms) that the internal resistance of the element furnishing the electromotive force, together with the resistance of the large copper connecting wires, practically disappears for ordinary measurements. As the fall of potential is uniform throughout the 20 meters, the difference of potential between post 0

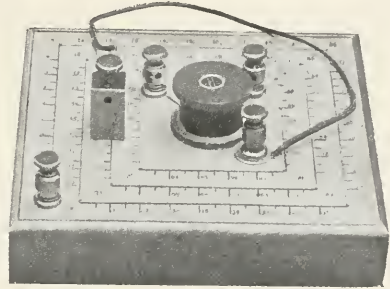


FIG. 2.

and post 1 will be practically  $\frac{1}{20}$  the electromotive force of the element. By moving the contact block from post 1 toward post 0, any desired fraction of this  $\frac{1}{20}$  may be secured. The under surface of the contact block is bevelled so that the metal touches the wire only with one edge; the opposite edge is supported by a piece of hard rubber. A flexible cable leads from the contact block to the binding posts shown in the foreground to the right.

These pieces of apparatus are manufactured for the Harvard physiological laboratory under Dr. Porter's direction. They may be obtained through him at very reasonable prices.

R. P.

**Popielski, L.** Ueber die Zweckmässigkeit in der Arbeit der Verdauungsdrüsen. Kurzgefasste Kritik der Verdauungslehre des Herrn Prof. J. Pawlow. Deutsch. Med. Wochenschr. No. 48, 1902.

The well known theory of Pawlow's that the digestive glands function in a purposive way, adapting the secretion to the particular kind of food which is

to be digested is strongly criticized by Popielski. The paper deals more particularly with the functioning of the pancreas, where this purposiveness is supposed to be shown in the most pronounced manner. From results of his own work and from results obtained in Pawlow's own laboratory Popielski shows that this gland does not act in accordance with the "purposive" theory. An example will indicate the sort of evidence which is brought forward in opposition to the theory. It is found that a food mass made up of spleen, which contains no amylaceous substances calls forth a secretion from the pancreas with a higher content of diastatic ferment than that produced by feeding bread. Similar observations for other digestive glands are recorded.

R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt,  
Harvard University Medical School, Boston, Mass.

**Scott, G.** Formalin or Other Fixing Vapor, Followed by Absolute Alcohol, as a Wet Method for Blood Films. *Journal of Pathology and Bacteriology*, 7: 131-136, 1901.

According to Brinckerhoff and Tyzzer and Professor G. Sims Woodhead this method gives excellent results:

1. Hold the wet film, wet side down, in the mouth of a wide bottle, half filled with ordinary 40 per cent. solution of formic aldehyde, for about five seconds.

2. Drop, still wet, film side downwards, into absolute alcohol. Leave fifteen minutes, or, if more convenient, as long as forty-eight hours.

3. Blot off excess of alcohol, and move cover-glass to a dry part of the blotting paper.

4. Immediately, before any drying occurs, drop on a few drops of eosin-methylene blue stain (Jenner's stain); cover with a watch glass; stain for two minutes, no longer.

5. Allow excess of stain to run off the cover-glass, and rinse at once in a bowl of distilled water.

6. Blot off excess of water.

7. Dehydrate very rapidly in absolute alcohol, merely dipping in and withdrawing as quickly as possible.

8. Wash off alcohol in first xylol rapidly; wash in second xylol; drop on fresh xylol.

9. Mount in xylol balsam.

Scott insists upon the importance of using only pure distilled water to wash off excess of stain, and cover-slips, which are quite free from acid. The blood film must not be allowed to dry at any stage.

J. H. P.

**Brinckerhoff and Tyzzer.** On Amphophile Leucocytogenesis in the Rabbit. *Journal of Medical Research*, 8: 449-495, 1902.

Ehrlich and others have held that the granular leucocytes are formed in the bone marrow. The results detailed

in this exhaustive study bring additional support to this view. The bone marrow is regarded as the chief source, if not the only source, of the amphophile leucocytes of the rabbit.

In the early stages of mild peritonitis, produced by the injection of a dilute suspension of turpentine, the amphophile leucocytes accumulate in the mesenteric vessels and in the surrounding tissues. Coincident with this the number in the peripheral blood decreases, but later increases, and the bone marrow becomes depleted of adult amphophile leucocytes.

The following method was employed in order to obtain satisfactory preparations of mesentery and omentum. The fresh mesentery was spread out over the end of a segment of wide calibre glass tubing, or better, the severed neck of

a wide mouthed bottle, and tied on by strong thread. The adjacent mesentery and attached intestine was then cut away and the bottle top, with its "drum head" of mesentery, dropped into the fixing fluid. The mesentery remained on the bottle top through all the steps of fixation, hardening and staining until the final clearing with xylol previous to mounting. A sheet of cigarette paper was then spread over the exposed surface of the mesentery and with a fine pointed scissors a square or circle of mesentery and of paper was cut out. The two were then laid on a slide with the paper uppermost. One application of a blotter would remove enough xylol from the paper to permit of its being seized with a fine pointed forceps and stripped off the mesentery, leaving the latter spread evenly on the slide. The mesentery was then treated with balsam and covered with a cover glass. By this method all distortion and wrinkling of the mesentery was avoided.

The adult amphophiles are formed from the undifferentiated marrow cells, which cannot be distinguished from lymphoid cells. They are of small size with a round vesicular nucleus and a moderate amount of protoplasm which contains a basophilic cytotreticulum. Every intermediate form is found in the marrow between these simple cells and the adult amphophile. In addition to the formation of amphophiles by differentiation of these simple cells they may arise by the multiplication of the myelocytes. The amphophiles do not multiply.

The supply of amphophile leucocytes in the circulation is kept up by the entrance of fully formed cells from the bone marrow. The hypothesis is advanced that the chemical composition of the blood serum exerts a chemiotactic influence on the leucocytes in the bone marrow and draws them into the circulation. A leucocytosis is only an indication of an increase of the chemiotactic substances in the blood.

J. H. P.

**Lang.** Ueber die Resistenz der rothen Blutkörperchen gegen hypoisotonise NaCl Lösungen bei Magenkrebs. Zeitsch. für klin. Med. 47: 153, 1902.

It has been shown that in icterus and infectious diseases there is an increased resistance of red blood corpuscles to hypotonic sodium chloride solu-

tions. Lang has devised a method of determining the resistance of red blood corpuscles against the hypotonic sodium chloride solution. The method depends upon the fact that as red blood corpuscles go into solution, the mixture becomes transparent. He studied seventeen cases of carcinoma of the stomach and twenty other cases of stomach disease, and found that resistance of erythrocytes to lowering of osmic pressure of the surrounding fluid is generally greater in cases of carcinoma than in other stomach diseases. He attributes this change to toxic products formed by the neoplasm. This condition becomes more marked as the disease progresses. The author suggests the hypothesis that a toxin formed by the new growth causes at first hemolysis, but later the red blood corpuscles grow more resistant to the toxin, and this same resistance acts against hypotonic solutions.

J. H. P.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn, Wesleyan University, Middletown, Conn.

**Kuhn.** Die Assimilation des freien Stickstoffes durch Bodenbakterien ohne Symbiose mit Leguminosen. Fühlings Landw. Ztg., p. 2, 1901.

The author gives an instance of a very practical demonstration of the importance of nitrogen assimilating organisms in the soil, independent of the action of legumes. It consists of observations made upon an experimental plot in the Agricultural Institute of Halle. For twenty years this plot has been under cultivation, producing every year the same crop. A study of the harvest has shown that there has been, practically, no diminution in the harvest, and that the amount of combined nitrogen in the harvest and soil has not diminished as the result of the twenty years cultivation. Inasmuch as no legumes were planted in the soil during the whole period and there was no diminution in fixed nitrogen, the author concludes that it must be because there are present in the soil some agencies, probably micro-organisms, which assimilate free atmospheric nitrogen.

H. W. C.

**Winslow, C. E. A.** Color Standards for Recording the Results of the Nitrate and Indol Tests. Inst. of Techn., Boston, Mass.

The study of the effect of external conditions upon *B. coli communis* has emphasized the need of some different standard by which the capacity of the bacteria to produce nitrate and indol can be quantitatively measured. Even when the conditions of experiment are rigidly controlled, striking variations sometimes appear, and the law of such variations can be properly studied only when the results are carefully compared with each other.

The use of a color standard for measuring the reduction of nitrate and the formation of indol obviously suggests itself as simple and more practical than any other.

Up to the point at which a precipitate forms in the nitrite reaction, the depth of color in both cases may be considered as roughly proportional to the amount of the end product formed by the bacteria in a given time. The problem for the bacteriologist is, then, to select from the numerous schemes of color values prepared for artistic and educational purposes that one best suited for the matching of the reaction in question.

The most rational system of color standards which has come to my notice is that prepared by Milton Bradley, of Springfield, Mass., based on pure spectral colors of known wave length. It is issued in the form of a small booklet, and by cutting out and pasting to a card the colors between Red and Yellow Orange and their tints, a chart is obtained on which the color of the indol reaction produced by *B. coli communis* can be readily matched. The hue is read by holding the tube parallel to a white surface and looking through it at right angles, while



the matching color on the card is isolated by a small card with a window cut in it. The tube and card are viewed in strong diffuse daylight.

The Milton Bradley color scheme has not, however, proved satisfactory for measuring the reduction of nitrates. A large majority of the tubes tested lay somewhere between the Reds and Violet Reds of the scale, and could not be well matched with either. I, therefore, sought for some other standards and found more suitable ones in the book of standard colors published by Louis Prang, Boston, Mass. This system has no definite scientific basis. The hues are less pure and the tints less bright and clear than in the Bradley system. The gradations, however, are more numerous. Of the seven plates in the Prang book, the last five, including the darker shades, are not needed. On the "pure color" plate and the "first shade" plate the colors produced by both the indol and the nitrate test can be quickly and easily matched. As far as this standard has been used, it has been found to be a satisfactory system of record and an important aid in forming definite ideas as to the behavior of micro-organisms under various conditions.

H. W. C.

**Stokes, William R.** An Inquiry into the Role of Domestic Animals in the Causation of Typhoid Fever. Maryland Med. Jour., 1900.

The author undertakes to determine how far some of the common domestic animals may be concerned in the distribution of the typhoid bacillus.

For this purpose he uses chickens, white rats, rabbits, guinea pigs, calves and pigs. In all cases he has fed the typhoid bacillus in considerable quantity to the animal, and at varying intervals has studied the fæces for the purpose of disclosing, if possible, the presence of typhoid bacillus. He finds that the typhoid bacillus disappears with extreme rapidity in the intestine of these animals, and in the fæces; from none of them is he able to isolate living typhoid bacilli. He concludes, therefore, that the dejecta of these animals play no very great part in the distribution of typhoid fever.

H. W. C.

**Lesage and Delmar.** Contribution à l'étude de la diarrhée des jeunes veaux. Ann. d. l'Inst. Part XV, 417, 1901.

The authors have made a bacteriological study of a disease more or less common among young cattle, which is

somewhat similar to what is known as "white scour." This disease has previously been studied by a number of bacteriologists, who reached the conclusion that it is produced by certain varieties of the common *B. coli*. These authors differ totally from this conclusion. They find, it is true, that the *B. coli* is almost always present in the cases of this disease, but the universal presence of this organism is no special argument, inasmuch as it is so widely distributed. They find, however, another form of bacterium present; a coccus, which they say belonged to a group called Pasteurella. This organism, from the very outset, they regarded as suspicious, and they instituted a series of careful experiments which convinced them that this, and not the *B. coli*, is the cause of the disease in question. This organism is capable of producing the disease in experiment animals, when properly inoculated, and is always found characteristic of the disease. The disease is somewhat rapid, proceeding to its crisis, sometimes in one to two days and sometimes in eight to fifteen days. It is especially characteristic of young cows, and is believed by the authors to find entrance into the animal by means of the umbilical cord, and thence into the blood. In animals slightly older the inoculation is chiefly through the nasal membranes. It produces a septicæmia of the blood, which progresses until usually it is eventually fatal. The authors have attempted to find some method of vaccination against this disease, but, although their results are promising, they are not, as yet, especially successful.

H. W. C.

## NEWS AND NOTES.

The biological laboratory of the Brooklyn Institute of Arts and Sciences, located at Cold Spring Harbor, Long Island, will hold its next regular session for six weeks, beginning Wednesday, July 1st. Courses are offered in high school zoölogy, by Dr. Davenport and Mr. Lutz: in comparative anatomy, by Dr. Pratt; in invertebrate embryology, by Dr. Sigerfoos; in animal bionomics and variation, by Dr. Davenport; in cryptogamic botany, by Dr. Johnson; in ecology, by Mr. Whitford; in bacteriology, by Dr. Davis, and in microscopic methods, by Mrs. Davenport. Fifty students are admitted to receive instruction, the tuition fee being \$25. A limited number of rooms are offered, free of rental, to properly qualified investigators. Application for such rooms, or for further information, may be made to Professor C. B. Davenport, University of Chicago.

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We recently received the Catalogue of Exhibits at the Sixteenth Annual Exhibition of the Department of Microscopy of the Brooklyn Institute of Arts and Sciences, held February 14, 1903. The attendance at the exhibition was between 300 and 400. In connection with the department the following historical note is of interest: The Brooklyn Microscopical Club was organized February 10, 1881. Meetings were held twice a month at members' houses. Between February and April, 1888, through Professor C. W. Peckham, it was merged into the microscopical department of the Brooklyn Institute. The one thing necessary for the success of a scientific institution, the publication of papers, either generally or selected, is not done except in connection with the museum. This was the first department of the institute and had sixty-eight members when formed. The department has 134 members now. It has a conference and lecture once a month.

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Mr. C. M. Clark offers the following note, which may be of interest:

"I find that immersion (cedar) oil can be perfectly removed from a lens by *distilled water*. To be sure, it requires a little more rubbing than when xylene, etc., are used, but then *there is no danger of damaging the lens setting*. I use a piece of any fine linen, which has been carefully washed and dried away from dust. The oil is first wiped off as far as possible with this; then a corner is wet with water and wiped once over the lens, which is then wiped dry with another portion of the cloth. About three such applications are generally necessary to remove all the oil, but I have repeatedly removed oil that I have been using for nearly an hour in less than three minutes. I suppose 'lens paper' would do just as well as linen, but I have never used it."

The same writer says:

"The hairs from the pods of cowhage (*Mucuna pruriens*) make interesting objects of study. With a power of about 500 it is easy to see the cause of their

peculiar penetrating and irritating action. I recently purchased some cowhage hairs for the purpose of mounting, and as I could only procure them in somewhat large lots, I have more than I can use. Should any of your readers wish to examine the hairs I shall be glad to share with them as long as my supply holds out. Of course, the serious consequences that would follow should any of the hairs get into the eyes, must not be forgotten when mounting."

TECHNIQUE IN STUDY OF BODY HAIRS OF LEPIDOPTEROUS LARVÆ. Am. Nat. xxxvi: 427.—Special histological methods were found necessary to successfully differentiate the exceedingly delicate nerves of touch. For general anatomy of the sense hairs the usual fixing and staining methods were used; but for staining the peripheral nerve fibers and cells the *intra vitam* methylen blue method was found especially useful. Grüber's B.x methylen blue,  $\frac{1}{2}$  per cent. solution in normal salt, was used. Injection by means of a small syringe was made usually in the side, back of one of last abdominal segments, and enough liquid injected to color the segments, near the head, care being taken to insert the canula only under the hypodermis and muscles and not into the alimentary canal. After injection the insect was left quiet—it must remain alive—for about three hours for most insects, but with some, as with *Pieris*, two hours was a better time, and four hours seemed best for *Datana* larvæ. After this period the nerves and nerve oells should be well stained and almost all other tissue free from stain. The specimen is then cut open lengthwise and pinned out over a hole cut in sheet cork, the muscles and viscera are removed by careful dissection, and then, on examining under a microscope, the nerves and nerve cells are seen stained upon the surface of unstained hypodermal layer. By keeping the preparations wet with normal salt solution and using different powers of the microscope, much may be learned regarding the structure and distribution of the peripheral nervous system. The silk worm, *Bombyx mori*, gave very good results for this work.

Such preparations fade in an hour or less and the ultimate distribution of the terminal nerve fibers cannot be seen without sectioning.

The following modification of Bethe's formula for fixation was used:

Ammonium molybdate	- - - - -	1 g.
Con. HCl.	- - - - -	10 drops
Dist. H <sub>2</sub> O	- - - - -	100 c. c.

This solution was used ice cold and allowed to act on the tissues from eight to twenty-four hours, after which they were washed well in cold distilled water and placed in absolute alcohol for about three hours, then cleared in xylene, and either mounted whole or embedded and sectioned.

## QUESTION BOX.

No. 30. What is the exact formula for Kyseline fluid?

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Edited by L. B. ELLIOTT.

**SEPARATES.**

One hundred separates of each original paper accepted are furnished the author, gratis. Separates are bound in special cover with title. A greater number can be had at cost of printing the extra copies desired.

IN the last issue of *School Science* Professor W. F. Ganong, in commenting upon an improvised apparatus for demonstrating osmosis, says :

"There are two other reasons why I think these shells, especially if used with calibrated tubes, are superior to such devices as those described by Mr. Adams, excellent though the latter are. First, they show the process in much greater simplicity than the carrot, for we are dealing with a single membrane and not with a complicated structure, the operation of which needs considerable explanation. Second, and more important, the experiment with the shells can be conducted with a precision and neatness far more illustrative of the real nature of scientific work than is possible with the make-shift devices. I have myself been a great advocate of home-made apparatus and make-shift methods, even going so far as to maintain that upon the whole such self-made arrangements are educationally superior to those that could be purchased especially made for the purpose. But increasing experience is making me change that opinion, and chiefly for the reason that these make-shift devices introduce the students to a wrong ideal of scientific work, and one which it is almost impossible for them to get rid of afterwards. The very soul of scientific experiment is precision and simplicity, or I might say precision, which presupposes simplicity; and it is most desirable that students shall receive this impression of it at the start. Make-shift devices are better than none, but the precise arrangements are best."

We have met many eminent educators who have held the view that the putting together of make-shift apparatus before the student gives a better comprehension of the causes and effects in the demonstration, hence a better understanding of the results. "Give me a piece of tin, some solder, wire, and a pair of shears, and I will produce almost any piece of laboratory apparatus," said one. Can there be anything more unscientific, more demoralizing to the receptive minds of students, than the laboratory tinker? Ingenuity is always to be commended, but it had much better be expended in devising new applications for well made and accurate appliances than in tinkering up new ones for the same purpose. The teacher or investigator who has a few good pieces of apparatus and is master of them finds little use for make-shifts.



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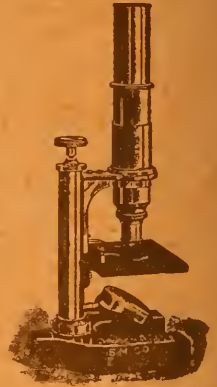
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## Laboratory Work in Zoölogy in the DeWitt Clinton High School, New York City.

I have been asked to write concerning laboratory work in zoölogy in the DeWitt Clinton High School. I can do this only with the understanding that I am describing the work that has been done, and not the work which it may be necessary to do to meet the requirements of the new syllabus recently published in the JOURNAL OF APPLIED MICROSCOPY. With a half year of zoölogy in a one year's course in biology, and part of that half year required for human physiology, there will be difficulty enough; but with zoölogy sometimes in the fall when insects are plentiful, and sometimes in the spring when there are none to begin with, one must believe that teaching the subject "under the best conditions obtainable," as the syllabus has it, will indeed be a matter for serious thought.

It has been shown by workers in the field of high school biology that good courses can be made up in a variety of ways. Pupils can be interested in a course on the crayfish alone; their attention can be held by the presentation of all the principal types, and it can be done with the types presented in any order whatever. The success of an elementary course in zoölogy depends largely upon the teacher, and upon the element of organization in the course. I mean by organization the careful allotment of time to each topic, the definite order of presentation of the important facts and their presentation in a way that will lead to comprehension. All this may be attended to and never a thought given to whether there is a *best* content of a course, or a *best* order of topics to be presented.

It may be that before we have come to some understanding on the question of the best content and the best order of topics of a course in zoölogy, the elementary teaching of the subject will have taken the final step on its way downward from the college into the elementary school. I believe that such a step is inevitable. President Eliot has recently called attention to the fact that at present we compel the pupil to wait till he is at least fourteen years of age before we give him an intelligent glimpse at the world with which he has long been in sympathy.



In New York city over 98 per cent. of the pupils who enter the elementary schools never enter the high school at all ; only 50 per cent. of those who graduate from the elementary schools enter the high schools. It looks very much like intellectual robbery to continue to deprive that great number of the many valuable ideas, well within their power of comprehension, which we now in a belated way teach to first year high school pupils. Another consideration more important, in a sense, than that, is involved in the fact that justice is not done to the sciences in furthering their usefulness in educating the people. It is time for the so-called laity to cease regarding the achievements of science at a distance, "marvelling at the wonders of the age," stupidly. A truly educated public, when it comes to exist, will no longer be content with feeding itself chiefly on the intellectual food of its own deeds in literature and politics, but will grow into larger being by the study of the relations existing in the universe, and the forces which underlie all things. For a long time, history, literature, and mathematics have had their foundation in the curriculum of the elementary school; none of the sciences have been adequately introduced there. Until that is done some knowledge of a science will continue to be regarded as an accomplishment rather than as an intellectual necessity. The cry of overcrowded curricula should have no influence in the matter; it is rather a question of what we need least. It is inevitable that the same "natural selection" should go on in the elementary schools that has been going on in the colleges since the time of the early realists.

While considering the developments of the future, it is urgently necessary to do for the present the best that circumstances permit. I think anyone acquainted with the teaching of biology in the high schools of the country will agree that a very substantial advance has been made within the past ten years. Every earnest teacher of the subject has contributed his part. Unquestionably more has been done in this time by the high schools of the West than by those of the East. In New York we awaken slowly, but once awakened we move with giant, if sometimes uncertain, strides, in educational as well as in other municipal affairs.

At the present time there are fifteen hundred boys studying biology and other first-year subjects in the DeWitt Clinton High School. When these boys come to us they are almost wholly untrained in the habit of mind essential to scientific work; when they go from the high school we can hope that the habits of concentration and discrimination have begun to be developed. The evidence of the useful employment of time is never more real than when found in work. I trust that the drawings sent with this article will serve as tangible evidence of what can be done by young boys working with a serious purpose. The content of our course, the order of the topics, and the allotment of time are indicated briefly as follows:

*Fall Term.* Insects, five weeks; Crustacea, three weeks; Annelids, etc., two weeks; Mollusks, two weeks; Echinoderms, one week; Coelenterates, one week; Protozoa, one week; Vertebrates, five weeks. Total, twenty weeks. The field covered is indeed a great one, but there must be some attempt to present the entire system of facts, otherwise the pupil will miss the conception of the variety of organic forms. Another reason for offering a considerable



number of forms is the lack of patience in the untrained pupil for long continuing detailed study. They want a change. It may be possible to repress this habit of childhood, but I do not think it is desirable to do so. We do not expect the pupils to become investigators. Our purpose is to enable them to become acquainted with the appearance of several of the important types of animal life; to examine the demonstration dissections of all the laboratory types; to understand that in the several organs, physiological processes are carried on; to see by experiments and explanations what a physiological process is, and the part

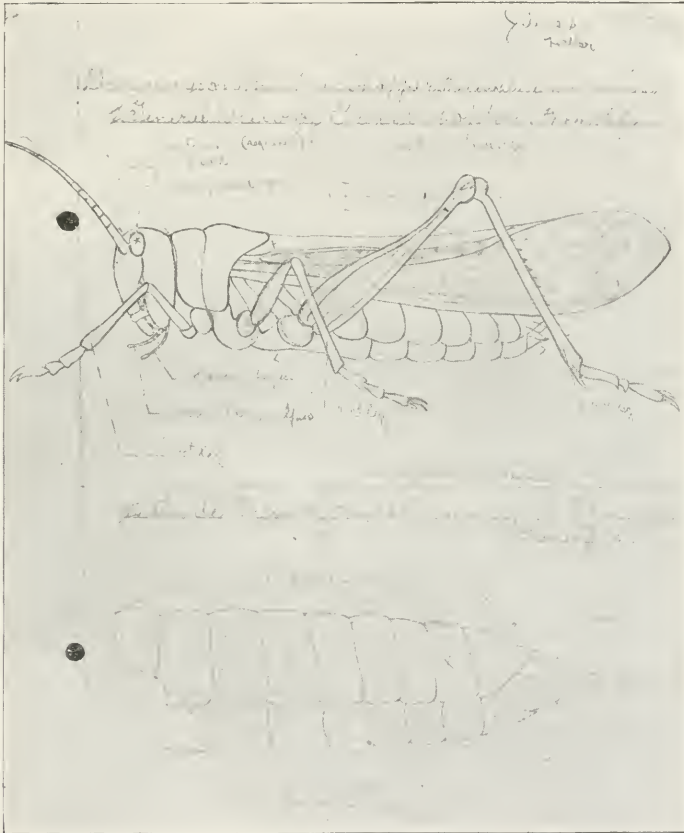


FIG. 1.

each one plays in the sum process of living. Whenever the opportunity offers, and that is very frequently, the pupil gathers into his increasing store of knowledge examples of adaptation. This is always an interesting field, because it explains things easily to him.

In beginning the study of a group, as the insects, or crustacea, we have a customary method of procedure, based on the belief that the pupil should obtain first of all some knowledge of the morphology of the organism. Whether this knowledge is superficial or thorough, it is something his mind holds and adds to in a way that it can not do if the animal is described for him in a book first, or

if a physiological process is explained to him before he has the organ in which the process is carried on, or before he has seen the process in actual operation, when such a thing is possible. Laboratory work, therefore, comes first. Our estimate of its relative importance is expressed in the fact that we give it, on the average, three of the five periods of the week. Our first laboratory study is the locust, *Melanoplus femur-rubrum*. This form is familiar, easily obtainable, and has definite external features; it leads immediately to the most valuable of all the groups for elementary study. On the first laboratory day of the term, the prepared specimens are passed around, one to each pupil. Attention is called to the nature of the questions of which copies have been given out. The questions are few in number, about ten to twelve, seldom more. They are simple and direct, dealing at first with one point only. A question is often preceded by a note which the pupil is expected to distinguish from his answer in the report he makes of the study. We find that numerous directions for the pupil to "note" this, that, or the other, are of very little use, because unless the pupil is held individually responsible for the correctness of certain feasible observations which he himself may make, he will grow into the habit of carelessness. He can very well assume that what the teacher or the manual tells him is there, is there of course, and he has no immediate concern with it. The notes we give deal with points which the pupil can not well make out for himself; but he is required to use the information immediately by answering a question bearing on the same point.

There is at once an advantage and a disadvantage in requiring answers to questions which suggest but one point. On the one hand, we can hold the pupil's mind to a definite thing, and compel him to discuss that one thing in a restricted way. For example, we may ask the question, "Are the antennæ jointed or not?" Here but one point is involved, and there is no reason for saying anything about the form or the length of antennæ. Another question might be, "How long are the antennæ?" This requires no word concerning the jointed condition of the appendage. On the other hand, these questions seem petty. They are, and the constant use of their kind becomes tiresome and mechanical. The questions should be sufficiently varied in form to encourage the habit of minute examination, and also the habit of grasping a larger idea in which may be involved matters of adaptation, for instance, the slender form of the antennæ, the thickness of the first wings, the thinness of the second wings, and the great relative size and strength of the thorax. (If the teachers of English could teach biology they might find excellent opportunities for natural drill in connected description or exposition. They are doubtless appreciative of the fact that some of this work is done for them by the teachers of biology. Let us hope it is well done.) A carefully prepared set of laboratory questions has a more decided physiological effect (sometimes called moral) upon a class of careless, indifferent, possibly disorderly, boys, than any "interesting" introductory remarks on the locust a teacher can devise.

While the boys are engaged in writing answers to the questions, the teacher may be doing a variety of things; he may sit quietly surveying the scene, or he may occasionally go the rounds of the class quizzing them gently, not caustically

as is the fashion in some places, or he may check what may prove a useless employment of the time of a pupil who puzzles over some meaningless detail, or finally, the teacher may read test answers in the report with a view to criticizing the accuracy, the clearness and the form of the answers. All these things can be done and done well with classes which do not exceed thirty-five in membership; with more than thirty-five the strain upon the teacher becomes too great, and the effectiveness of the class work correspondingly less. When the report on the locust is finished it is set aside, and preparation is made for the drawing. No drawing or sketching is permitted in the report, because we believe the pupil should recognize the value of being able to tell about something without the necessity of making a sketch to show what he means. The

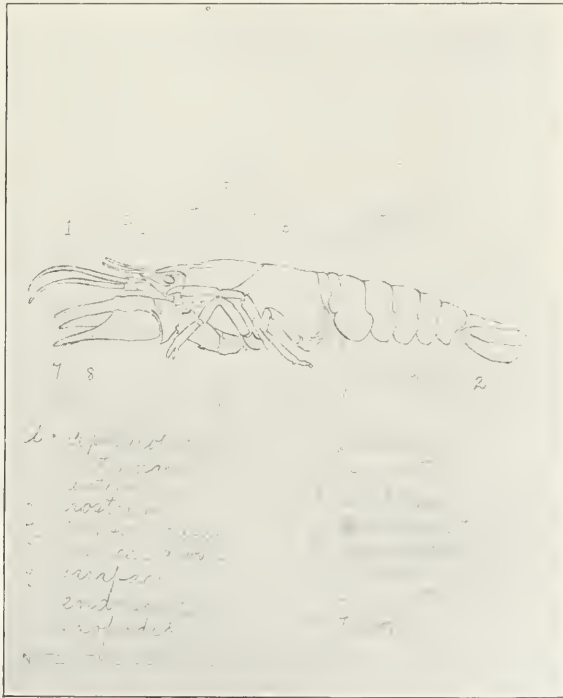


FIG 2.

energy that might be dissipated through a half-dozen sketches is saved for one very formal and complete drawing of the lateral aspect of the insect. The effect of this policy may be appreciated, when we remember that a good drawing is far more impressive than any number of diagrams, and indeed, quite as valuable in its reactive influence, as a well written description.

We consider time well spent if two forty-five minute periods are taken to complete a general drawing, approaching in excellence the one shown in Fig. 1 (p. 2303) of the locust. Of course, we usually are obliged to accept results much less satisfactory than those indicated. While the boys are engaged in drawing, the teacher's time is fully occupied with individual criticisms (class criticisms do

very little good) in proportion, definition, and the relative importance of lines. I do not believe that any boy, if permitted to go as he pleases, would ever learn the important fact that the sum of the details makes the whole. One of the most difficult things in teaching draughting is to lead the pupil to recognize the importance of details as they contribute to the conception and representation of the entire organism. It is a mistake for the teacher to draw on the blackboard a diagram of an animal for the purpose of showing the pupils what he wants. They can be told what he wants; the execution of the task is something for which they should be held responsible. The first attempt at scientific drawing is likely to prove discouraging to a young boy if he feels the standard pressing him closely. He is apt to say that he "never could draw anyway." I find it helpful to show the work of a good draughtsman in the class, as an example of what can be done. The inefficient pupil can usually be convinced of the fact that his failure is due to some particularly designated oversight in execution, and not to a constitutional inability to draw. When the drawing is done, all the important organs are indicated, best by numbers with an explanatory table written at the left lower corner of the page.

It is difficult, in the time we have, to find a suitable opportunity for demonstrating dissections which the pupil is not skilled enough to investigate for himself. As a rule we use one period about every second week for reference reading, at which time the pupils are supplied with books different from their regular text-book, and required to read and abstract. During this period demonstrations of dissections can be made to two or three pupils at a time till the entire class has been called up.

Two periods are given to the study of the activities of the locust. In the country high schools this study could be undertaken independently by the pupils outside the regular school time; in the city it is of course impossible. Inverted glass tumblers with a blade or two of grass is all the apparatus needed to make satisfactory observations on the *locomotion*, *feeding*, and *breathing* of any species of locust, grasshopper, or cricket. An excursion to the country in September, begun in the afternoon in the time that would otherwise be regular school time, has always proved invaluable to us in making it possible for the boys to get acquainted with things and conditions in general in nature. The difficulty of accomplishing such an undertaking from the region of Fourteenth street, New York city, is probably as great as one would find in any city in America. After the work on the locust is completed, we devote a few periods to the butterfly and the bee, or to the beetle, the method introduced with the locust being continued in abbreviated form. Recitations on the group of insects complete the first five weeks' work.

The crayfish is the form we take to represent the Crustacea. One prepared specimen is issued to every two boys. The questions on the crayfish require comparison with the locust, and introduce in a simple way the principle of homology. Adaptations of form to function are plentiful in the crayfish, and are easy for study. The drawing of the crayfish is probably more difficult to do than that of the locust. One of the best drawings of the crayfish done this year in the school is shown in Fig. 2.



Work with the living crayfish is always profitable, and I might say, boisterously interesting. We employ glass and porcelain-lined trays ten to fourteen inches long and two and one-half inches deep, filled with water. With an active crayfish in one of these, two pupils working together can observe definitely the mechanics of locomotion; by the aid of carmine poured upon the upturned ventral surface the manner of flowing and the rapidity of the current which passes through the gill chamber can be seen, as the animal is returned to the water; responses of the sense organs to stimuli can be obtained without the use of corroding chemicals. This study may well take two periods to complete.

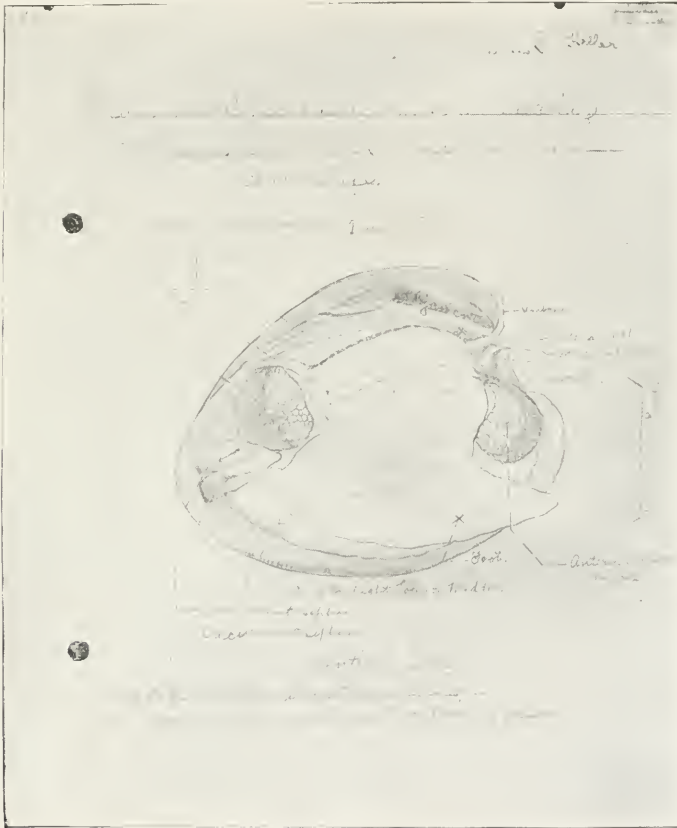


FIG. 3.

We have recently inaugurated a plan for the presentation of the facts of nutrition, with a view toward leading up to the understanding of human physiology. The problem of the correlation of human physiology with elementary zoölogy is by no means as simple as it is sometimes supposed. Scientifically, human physiology has no more place in a course of elementary zoölogy than comparative embryology has. By uniting elementary zoölogy and human physiology we comply with a State law in reference to the teaching of physiology, and also save time for some other subject in the high school curriculum.

The demonstration dissections of the locust and the crayfish give the pupils some knowledge of the way food is disposed of. Ingestion and digestion are discussed and explained by lectures, experiments and recitations. Application of what is learned in that way is made to the more complicated conditions in the human digestive organs and physiological processes. At some time in the two weeks given to the annelids, the next two stages in nutrition are explained. The earthworm is unusually favorable for simplifying the ideas of absorption and circulation. The intestine is a simple tube, and the circulatory system is clearly

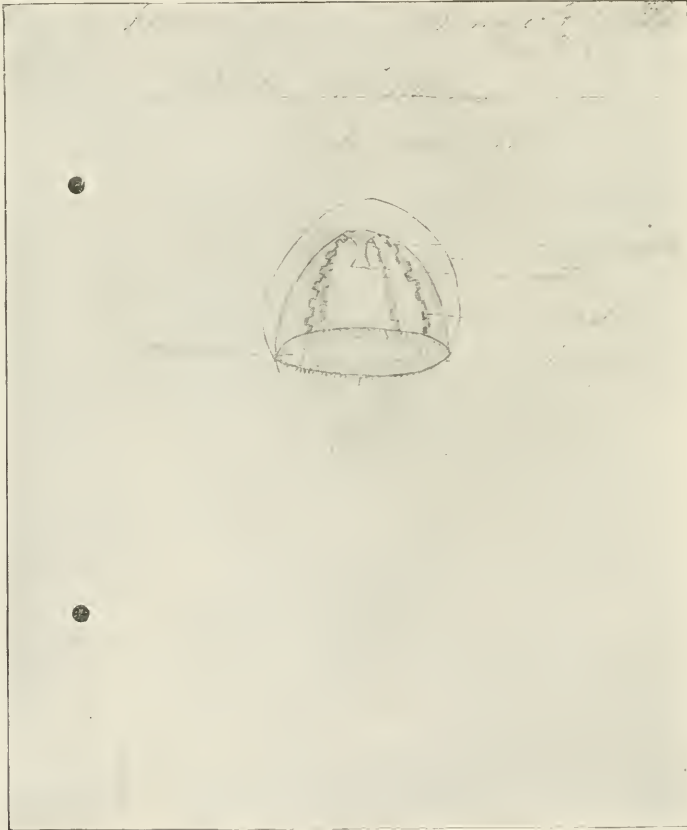


FIG. 4.

defined. Experiments on osmose are made simple to help in explaining absorption in the time we have set aside for the clam.

Tracheæ, gills, and and lungs are then discussed as organs which coöperate with the circulatory system, to transport liquid and gaseous food to the tissues. Assimilation, or the synthesis of protoplasm, must, because of its nature, be left only partially understood. Oxidation and the release of energy are, I am sure, never thoroughly comprehended by a beginner. The idea is too complex for one with no intellectual perspective. However, our faith in the usefulness or

teaching *at* the idea is shown by the fact that we recur to it time and again in zoölogy and also in botany.

I believe the relation of oxidation to the process of excretion is not made clear by most text-books. That should be done. Moreover, it is essential that the pupil should understand not only that nephridia, kidneys, and skin are excretory organs, but that tracheæ, gills, and lungs are, also. When we come to the Protozoa, we find a good opportunity to make a point of the fact that all the processes of nutrition may be carried on in one cell. I may say in passing that we use the compound microscope in the study of Protozoa. That is the only time the class as a whole uses it in zoölogy. Very little is said about physiology while our time is occupied with the starfish and the hydroids.

During the five weeks study of the frog, the pupils come into a fair understanding of the anatomy of their own bodies, by comparison, and of the physiological processes going on there. The frog is the only form that the pupils dissect. They do it with considerable satisfaction and profit. Our course as it has been planned in the past requires us to lay in a stock of frogs in the middle of the winter. This is expensive, but the difference of a few cents for each pupil is of no consequence in comparison with the benefit obtained from the careful individual study of so good a type, at the close of their all too brief introduction to zoölogy.

HENRY R. LINVILLE.

DeWitt Clinton High School.

## Demonstration of Phototropism in Aquatic Plants.

At the close of the school year last June, an aquarium jar of fresh *Philotria* (*Elodea*) was covered over with a glass plate to preserve it, and placed on the sill of a south-facing window. The jar was not touched until the September following. When it was examined, the stems of the still vigorous plants were found to be nearly parallel, and with all the tips extended upward and toward the source of light. The reaction of mustard seedlings to the same stimulus is not more marked or beautiful.

C. STUART GAGER.

N. Y. State Normal College.

## Methods for Growing Pure Cultures of Algae.

The lack of some means whereby algæ, particularly the unicellular forms, can be successfully isolated and cultivated in a pure state, has led to some most remarkable theories with regard to the life history and affinities of certain species. The tendency for one to become an ardent "polymorphist," after studying the contents of a small jar from a single locality, was perhaps excusable at one time, but that such crude methods should be retained and serious morphological and systematic work attempted under such conditions hardly seems necessary in the present day. The following described media have been employed in an attempt, still in progress, to monograph the unicellular green algæ, where

the importance of having absolutely pure culture, without any possibility of contamination, cannot be over-estimated. The only excuse for publishing them at this time, rather than in connection with the investigation with which they properly belong, is that the numerous requests for some artificial medium to be used in growing algæ makes it seem probable that a wider use of certain methods may lead to more definite and satisfactory results in the study of these forms, and that our knowledge of them may be rapidly and accurately increased. It should be said at once that there is no one method or medium which is equally well adapted to all algæ. The slightest modification in concentration or ingredient is often sufficient to prevent luxuriant growth, and the advantage of special formulæ for certain groups and genera becomes more and more evident as one's experience increases.

Beyerinck was perhaps the first who successfully attempted the growth of algæ in artificial media. His earlier method was to use sterilized ditch water with 10 per cent. gelatine, a drop of water containing the algæ to be studied being added just before the mixture hardened. The chief objection to this nutrient solution, aside from its uncertainty and indefiniteness, is the great difficulty of properly sterilizing the ditch water. Spores of certain blue green algæ are tremendously resistant to even boiling temperature, and the constancy with which one gets a blue green culture after having inoculated with a grass green organism is calculated to produce a certain degree of patience with the authors of such articles as "Algæ, the Spawn of Mosses," etc. A modification of the ditch water method, wherein the gelatine has been previously liquified by pancreas, is supposed to induce more luxuriant growth, but it seems to possess no particular advantage over the unmodified gelatine.

Perhaps the best known artificial medium for growing algæ is that devised by Knop and frequently referred to in subsequent literature. The formula for this, as generally given, is:

4 parts calcium nitrate,  
1 part magnesium sulphate,  
1 part potassium nitrate,  
1 part potassium phosphate.

It is much better, however, to prepare 10 per cent. solutions of all these salts, and obtain the desired dilutions before adding the calcium nitrate to the other three. In this way the insoluble precipitate formed by the calcium phosphate will be reduced to a minimum. Solutions containing from .1 to .5 per cent. of the magnesium and potassium salts, with four times as much calcium nitrate, are suitable for the cultivation of algæ, but higher concentrations may be used with some forms. Unfortunately, this solution, which is comparatively well known, is perhaps the least well adapted for growing many species, particularly in connection with a solid medium.

The source of nitrogen is naturally of considerable importance, it being particularly necessary in growing blue green algæ. and calcium nitrate does not seem to be the most suitable salt for this purpose. A much more satisfactory solution, which is a modification of another of Beyerinck's, is:





Forty drops of *A.* and twenty drops of *B.* are added to one liter of water in which five centigrams each of sterilized wheat straw and moss has been placed. In place of the wheat straw, 25 gr. of calcium silicate, precipitated and washed, may be added, and the sodium and potassium nitrate can be omitted without any marked effect.

Marine diatoms can be grown in agar made up with sea water, or the following synthetic solution :

Magnesium sulphate	-	-	-	-	-	-	-	-	2. gr.
Magnesium chloride	-	-	-	-	-	-	-	-	4. "
Distilled water	-	-	-	-	-	-	-	-	1. lt.

A few drops of solutions *A.* and *B.* may also be added with advantage.

Of course, all the above solutions may be used as liquid cultures, but as there is great difficulty in following a single organism by this method, it is frequently best to add either agar or gelatine, as most of the algæ are readily grown on a solid medium of this kind. Since it is desirable not to have the culture any stiffer than necessary, 0.5 per cent. agar or 5 per cent. gelatine is quite firm enough, if carefully handled. The agar should always be as free from organic matter as possible, this being accomplished by soaking in 1 per. cent. acetic acid for fifteen minutes and then rinsing thoroughly in distilled water. When it is desirable to use a medium absolutely free from organic matter and yet of a jelly-like consistency, there is nothing so satisfactory as the silica jelly recommended by Winogradsky for isolating certain nitrifying bacteria. As the method for making this medium is not generally well understood, most of the published accounts being incorrect or so meagre that it is impossible to manufacture the jelly successfully, the following details are given :

A volume of sodium silicate (specific gravity, 1.07 to 1.1) is added drop by drop to an equal volume of hydrochloric acid (specific gravity, 1.10), meanwhile keeping the hydrochloric acid well agitated. This mixture is dialized until there is no reaction for chlorine with silver nitrate. A perfectly clear liquid, without the slightest opalescence, that will stand sterilization to 115° C., should result. The reason for the general failure to successfully make this medium is usually due to one of the following causes :

1. Impure sodium silicate. The soluble glass must be absolutely transparent. If otherwise, it will begin to harden in the dializer, or will be so unstable as to make proper sterilization impossible.

2. Incorrect specific gravity. This should always be 1.10 for the hydrochloric acid. The specific gravity of sodium silicate may vary from 1.07 to 1.10, according to the make and consistency used. When commercial soluble glass is bought in liquid form, a specific gravity of 1.07 is about correct. If crystals are used and dissolved in hot water, the specific gravity will vary somewhere between 1.09 and 1.2.

3. Imperfect dializer tubes. It is absolutely necessary that the dializing membrane be free from holes, and at the same time as thin as possible, to permit the most rapid dialization. For this reason the collodion tubes described by Mr. Kellerman in the JOURNAL OF APPLIED MICROSCOPY AND LABORATORY METHODS (Vol. v, p. 2038, November, 1902) have been used with much suc-

cess. In any case, the tubes should be carefully tested by filling with water and being allowed to stand for some time.

4. Incomplete dialysis. It is usually sufficient to dialize in tap water for twenty-four hours, then in distilled water, changed several times, for the same length of time. Only small quantities (100 c. c.) should be dialized in one tube and the silver nitrate test always used.

Careful attention given to all the above points will result in the production of a perfectly transparent liquid, which, after sterilization, may be kept for considerable length of time in a cool place, if closely stoppered. Upon the addition of the nutrient salts desired, the fluid will harden in an hour or two, or if placed in the steam sterilizer, coagulation will take place in a short time. This medium is particularly serviceable in growing blue green algæ, and by careful selection of nutrient salts, a solution may be obtained which is so unfavorable to either fungi or bacteria that a pure algal culture may be maintained, even though subject to occasional sources of contamination.

In addition to the ordinary methods of growing algæ upon solid media, Chodat and others have recommended the use of pieces of half baked porous porcelain. These pieces, after being sterilized by dry heat, are put in Petri dishes partially filled with nutritive solution, and inoculations may readily be made by means of a sterilized pipette. The advantage of such a method is that algæ, which require thorough aëration, thrive well on the porcelain, but as a rule all organisms develop very slowly when grown in this way, it being sometimes six or eight weeks before the colonies appear.

For general purposes it has been found that Erlenmeyer flasks of about 100 c. c. capacity are the most suitable for growing cultures. These, when filled with nutrient agar to a depth of from 1 cm. to 3 cm., and tightly plugged with cotton, will last for a long time. If it is desirable to retain the cultures for future inoculations, the cotton can be covered with the thin rubber caps which come for the purpose, and thus the algæ will be kept alive for years without the risk of contamination. When large jars or bottles are used for cultures, the insertion of a small vial partially filled with sterile water, which will be held in place when the agar hardens, is of considerable service in insuring a moist atmosphere and preventing the drying up of the medium.

As a rule, the necessity for plating out a gross culture of algæ is not so imperative as with bacteria. The color and size of the algæ enable one to select a single plant under the microscope with which to inoculate, and the large amount of time saved in this way makes it undesirable to resort to other methods unless necessary. Repeated dilutions with a final and thorough examination under the microscope, is usually quite sufficient to insure a pure culture. It is even possible in this way to obtain cultures from single zoöspores, and while one cannot always be certain that the culture will grow, a sufficient number of dilutions and care in the use of perfectly clean pipettes will usually result successfully.

Marshall Ward, in the *Annals of Botany*, Vol. xiii, pp. 563-566, gives some ingenious means of separating gross cultures, which may be resorted to when necessary. One of the most satisfactory, and often used with success in separating

blue greens, is to mix the algæ with the nutrient solution before adding it to silica jelly; then, after a thorough shaking, the jelly is poured into shallow dishes to harden. The methods given by Ward, involving the use of plaster of Paris, and also a solution of lime water, which is precipitated by carbon dioxide and the calcium carbonate poured out, are decidedly too severe for the more delicate algæ one usually wishes to cultivate. It is generally much better to make a large number of cultures after repeated dilutions than to depend upon getting a pure culture from which to inoculate by plating out in one of the ways mentioned.

As was said in the first place, it is impossible to give any one formula that is equally well adapted for growing all algæ, and it is necessary to experiment in order to discover just the combination of salts, together with their strength, that will give the maximum results for the particular form under investigation. Still, it is often desirable to have a stock medium that will give fair results without requiring too much manipulation, and for this purpose there seems to be nothing quite so satisfactory as the modified Beyerinck's solution, using ammonium nitrate, and adding about 2 per cent. glucose. Other sugars are of benefit, but glucose seems best adapted for most unicellular algæ. This solution may be used with silica jelly, but is fully as satisfactory as for general cultures when hardened with from  $\frac{1}{2}$  to 1 per cent. agar. GEORGE T. MOORE.

Bureau of Plant Industry.

### A Substitute for a Microtome.

A short time ago a small piece of tissue was sent to me for diagnosis by a physician. The case was one of suspected malignant disease, which, if it proved to be such upon microscopic examination, should be operated upon immediately.

The piece was small, so I put it in 95 per cent. alcohol, changed the alcohol once and cut it the next day. As I have no microtome I intended to cut the sections free hand, but happened to think of a pair of straight, smooth compression forceps among my surgical instruments. By firmly holding the tissue about midway between the two ends of the forceps and letting the razor glide over the forceps I cut off the rough edge of the tissue. After this I carefully elevated the tissue very slightly above the forceps and found that I could cut a very creditable section. By exercising care several good sections were made. They were stained, cleared with clove oil and examined in the oil.

The diagnosis was made without any of the ordinary embedding materials, microtome or section razor. The only reagents and instruments used were alcohol, staining solutions, clove oil, an ordinary shaving razor, compression forceps and microscope. RAYMOND C. REED.

Elmira, N. Y.



## Demonstration of Fibro-Vascular Bundles.

Partly dead leaves of the Agave have been found especially good for this demonstration. Petioles of plantain, Calladium, or celery, or dried corn stalks are very commonly used for this purpose, but when these are not available, the partly dried leaves of small Agaves may be used to advantage. Fresh leaves will answer still better. After the epidermis of the leaves has been cut around, the leaf may be pulled apart. The fibro-vascular bundles will then appear as fine silken fibres, resembling threads of cobweb, much as is seen in Calladium petioles, only more numerous. By placing a length of the leaf in a solution of eosin or red ink the usual demonstration of these bundles as the path of the water current may be made.

C. STUART GAGER.

N. Y. State Normal College.

## A Review of the Methods of Staining Blood.

### VIII.

#### D. *Neutral Stains*.—Continued.

*Argutinsky* (1901) recommends the following modification of the Romanowsky-Nocht stain for staining malarial blood: Two stock solutions are made up,—

*a.*  $\frac{1}{10}$  per cent. eosin.

*b.* Soda-methylen blue, prepared by heating 48 hours in a warm oven (55°–60°C.) 100 c. c. of 1 per cent. methylen blue with 6 c. c. of 5 per cent. solution of soda.

Three cubic centimeters of the soda-methylen blue solution are mixed with 42 c. c. of distilled water. In another dish 5 c. c. of the  $\frac{1}{10}$  per cent. solution of eosin are mixed with 25 c. c. of distilled water. The two diluted solutions are then slowly stirred together. Blood films are placed in this staining solution for 15 to 20 minutes. The metallic film which forms on the surface of the stain is removed with filter paper before the preparations are taken from the staining dish. The preparations are washed 1 to 2 minutes in a series of dishes of distilled water, and after drying between filter paper are mounted in balsam.

*Argutinsky* also employs the same stock solutions undiluted with subsequent differentiation. In this case the stock solutions are allowed to ripen at least 5 days before use. To 15 c. c. of the 1 per cent. soda-methylen blue solution, 6 c. c. of the 1 per cent. eosin solution are added and thoroughly mixed. The metallic film on the stain should be removed with filter paper before the preparation is removed from the stain. It is then rinsed in water and placed in the differentiating fluid (120 c. c. of 95 per cent. alcohol, to which 4 or 5 drops of glacial acetic acid and 2 c. c. of 1 per cent. aqueous solution of eosin have been added). The preparation is moved to and fro in this solution, the superfluous

stain coming off in blue clouds, the preparation changing from a violet to an eosin red. Before the last trace of violet is washed out, the slide should be removed to water and thoroughly washed till no more color comes off. It is then dried between filter paper and mounted in balsam.

Several hematologists have recently separated the active staining ingredients of the Romanowsky neutral stain and have improved and simplified the technique of staining.

*Jenner* (1899) used equal parts of a 1.2 per cent. to 1.25 per cent. solution of Grüber's water-soluble eosin, yellow shade, in distilled water, and of a 1 per cent. solution of Grüber's medicinal methylen blue in distilled water mixed in an open vessel—not in a flask—and thoroughly stirred with a glass rod. The mixture is best left for 24 hours. It is then filtered and the residue dried either in the air or more quickly in an incubator or water bath. When quite dry the residue is powdered, shaken up with distilled water and washed on a filter. It is then dried again and powdered. For use thoroughly shake up 0.5 grams of the powder in 100 c. c. of pure methyl alcohol (E. Merck, "for analytical purposes") and filter. The solution keeps well.

A few drops of this fixing and staining solution are poured upon the dry blood and covered with a watch-glass to prevent evaporation. After 1 to 3 minutes' staining the solution is poured off and the preparation at once rinsed in distilled water until the film has a pink color, which usually takes from 5 to 10 seconds. The preparation is then dried and mounted in xylol balsam.

The red corpuscles are stained a terra-cotta color, the nuclei blue, blood platelets mauve, granules of the polymorphonuclear leucocytes and myelocytes red, granules of the mast cells dark violet, bacteria, filarial and malarial parasites blue.

*Leishman* (1901) has modified the methyl alcohol method of Jenner, and has secured a greater differentiation of the blood elements. His staining solution is made as follows:

Solution A,—Medicinal methylen blue (Grüber's), 1 per cent. solution in distilled water, to which is added 0.5 per cent. of sodium carbonate. Heat the solution to 65°C. in a paraffine oven for 12 hours, then let it stand at room temperature for 10 days before use. Solution B,—Eosin extra B. A. (Grüber's), 1 to 1000 solution in distilled water. Equal volumes of A and B are mixed in an open vessel and allowed to stand for from 6 to 12 hours, being stirred from time to time with a glass rod. The abundant flocculent precipitate which results is filtered off and washed with distilled water until the washings are colorless or only pale blue, then dried and powdered. This greenish powder with a metallic lustre constitutes, or at least contains, the active staining ingredient in Romanowsky's method. The powder is to be dissolved in methyl alcohol (Merck's "for analysis") in the proportion of 0.15 per cent. This solution does not deteriorate by keeping.

Three or four drops of this alcoholic solution are allowed to fall upon the blood film, and are distributed over the whole surface by gently tipping and rotating the cover-glass. No attempt is to be made to check evaporation. After about half a minute, double the quantity of distilled water is added and

allowed to mix with the alcoholic solution of the dye. The film is then allowed to stain for 5 minutes, then gently washed off with distilled water, and a few drops of the water allowed to rest on the film for 1 minute, when the preparation is ready for examination, either directly in water or after drying and mounting in balsam. "The part of the procedure in which the stained film is allowed to soak in distilled water for a minute after staining and washing has a triple importance. It intensifies the Romanowsky staining, it removes the remains of the deposit, and it alters the tint of the red blood corpuscles from a greenish blue to a transparent pink."

Red blood corpuscles are stained pale pink or greenish, semi-transparent. The polymorphonuclear leucocytes (neutrophiles?) have a nuclear network stained a deep ruby red color with sharply defined margins, extra-nuclear protoplasm colorless, granules red. The eosinophile leucocytes show a ruby-red nucleus and pale pink granules. Mononuclear leucocytes have the nucleus ruby-red with extremely sharp, clear outlines; extra-nuclear protoplasm eau-de Nil or blue, occasionally showing a few red granules. Lymphocytes the same as the mononuclears, except that the nuclei are, as a rule, more deeply stained. Basophile leucocytes have the nucleus red, but usually more or less marked by granules overlying it; granules very deeply stained a deep purplish black. Nucleated red corpuscles have the nucleus stained almost black, with a sharp outline; extra-nuclear portion gray. Blood plates are deep ruby-red color with spiky margins, frequently showing a pale blue peripheral zone surrounding the red center. Malarial parasites stain blue and their chromatin ruby-red; in case of the tertian parasite Schüffner's dots are well marked in the containing red corpuscle. Bacilli are stained.

Reuter (1901) worked on a similar line as Leishman and also succeeded in isolating the active staining ingredient of the eosin-methylen blue compound and applying it as a single stain. He also used absolute alcohol as a solvent of the neutral dye, a separate process of film fixation occupying at least an hour, and his staining process in itself occupies 2 or 3 hours.

Massachusetts State Board of Health.

ERNEST L. WALKER.

## Methods in Plant Physiology.

### XI.

#### XI. GEOTROPISM.—Continued.

9. **Effect of Different Media upon Geotropic Curves.** As has been shown by Nemec<sup>1</sup>, the shape of the curve of an inverted root depends to a large extent upon the nature of the medium in which it is grown. Figure 14 shows some of the curves which may be produced by different media.

Raise seedlings of *Lupinus albus* or of *Vicia Faba* in wide crocks of moist sand or sawdust; when they have attained a length of 3 to 5 cm. invert the crocks and allow the seedlings to grow for two days in that position. Employ seedlings of the same kind for experiments with gelatine, moist air, and water.

<sup>1</sup> Nemec, B. Jahrb. f. wiss. Bot. Bd. 36, p. 80.

Make a 7 per cent. solution of gelatine, dissolving the gelatine on a water-bath; when the solution cools to  $20^{\circ}$  to  $30^{\circ}\text{C}$ . insert several straight seedlings, allow the jar to stand until the gelatine hardens and invert it. Insert other



FIG. 14.—Roots Inverted in Different Media. No. 1 in moist air; No. 2 in water; No. 3 in moist sawdust.

seedlings in the cork of a wide mouthed bottle or pint jar at least 10 cm. in diameter. When the jar is inverted there should be an air space of 2 to 3 cm. above the surface of the water in order that a sufficient supply of oxygen may be

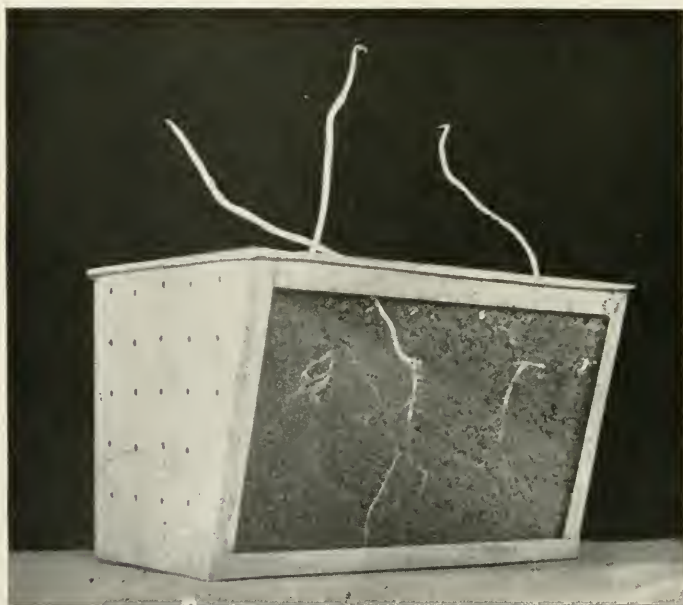


FIG. 15.—A Culture Chamber with Sloping Glass Side.

present. Other seedlings may be inverted in the damp chamber for 36 to 48 hours.

10. **Diageotropism of Roots.** The diageotropic, or plagiotropic, growth of



roots is best observed when the roots are allowed to grow against glass. The preparation must be kept in the dark if the roots are to follow the glass.

Fill a box with sloping glass side with moist sawdust and plant against the glass three seedlings of the pea (*Pisum sativum*) with only the main root grown (Fig. 15). Place one seedling in the normal position, one in the inverted position, and one in the horizontal position, all with the roots showing against the glass.

When the lateral roots appear their orientation may be observed and compared with that of the main root. After this observation has been made, stand the box on end and notice how the primary, secondary, and tertiary roots orient themselves with respect to the attraction of gravity.

## XII. HELIOTROPISM.

1. **Heliotropism of Stems and Roots.** When exposed to the one-sided action of light, the hypocotyls and roots of seedlings afford a good means of studying the heliotropic responses of the different parts of plants. Fasten four or five seedlings of white mustard (*Sinapis alba*) or buckwheat (*Fagopyrum esculentum*) 3 to 6 cm. long to a small wooden bar by means of blotting paper and rubber bands, and suspend them in a rectangular glass jar. Instead of lining the jar with filter paper, fill it with water up to the lower surface of the bar supporting the seedlings. The three sides and top of the jar are to be rendered opaque by a covering of black paper; or, better still, set the jar containing the seedlings into a box, the interior of which is painted a dead black, and which has a window on one side for the admission of light at the level of the seedlings. By means of the latter method one is absolutely certain that all side and top-lights are excluded. The open side of the jar is exposed to a bright sky, but never to direct sunlight. If the sky is very cloudy, an artificial light may be used as a source of stimulation, but should not be so close that the heat produced by the light will affect the plant.

The hypocotyls will show a strong, positive curvature, while the roots will be curved negatively.

2. **Latent Period.** Raise in the dark room a small crock of seedlings of German millet (*Setaria Italica*). When the seedlings are 1 to 2 cm. above the earth, if all are vertical, make rapidly the following preparation: Thrust two pieces of soft iron wire into the earth, one on either side of a straight seedling, bringing the seedling and wires into the same vertical plane. Set the crock into the box which admits light only on one side. Make the observations for curvature at intervals of ten minutes.

Conduct the experiment at the same temperature as in the test for latent period with geotropism and compare the length of the periods obtained with the two stimuli.

3. **After Effect.** Allow the plant used in the preceding experiment to acquire a curve of  $20^{\circ}$  to  $30^{\circ}$ , then readjust the two wires in such a way that they have the same inclination as the plant under observation, and revolve the crock horizontally through an angle of  $180^{\circ}$ . Observe at intervals of five minutes to

determine how long the shoot continues to bend in the same direction as before, determine also the time when the shoot begins to retrace its path.

Which of these two periods is the After Effect? Which is the Latent Period in this experiment? How does it compare with the Latent Period in the preceding experiment?

4. **Effect of Light Waves of Different Length.** Prepare two jars of mustard (*Sinapis alba*) or buckwheat (*Fagopyrum esculentum*) seedlings as in Experiment 1. Place one jar in a heliotropic chamber admitting only orange-colored light, the other in a chamber admitting only blue light.

Figure 16 shows a simple heliotropic chamber fitted with a chromatic window. The inner surface of the box is painted dead black, a tightly fitting door is made on one side of the box and a small opening cut in one end. The chro-

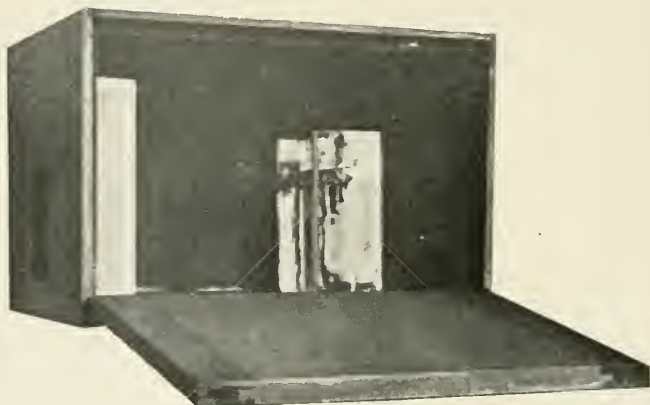


FIG. 16.—Heliotropic Chamber Fitted with a Chromatic Window.

matic window is made as follows: Select a large glass bottle with smooth parallel sides, cut holes in the cover and bottom of a cigar box of such a size that they will transmit no light except that which passes through the walls of the bottle when it is placed inside of the box. Pack the bottle tightly in the cigar box with black wadding and place it before the opening in the end of the chamber. In order to obtain orange light, fill the bottle with a saturated aqueous solution of potassium bichromate; to obtain blue light, fill the bottle with a solution of ammoniacal copper sulphate.

Observations should be made and the experiment ended within twenty-four hours. The short blue waves of the spectrum will invariably produce the greatest curves. Compare the results of this experiment with the light waves effective in carbon assimilation.

HOWARD S. REED.

University of Michigan.

## The Technique of Biological Projection and Anesthesia of Animals.

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### XIV. THE ANESTHESIA OF ANIMALS.—Concluded.

VERTEBRATA: *Frogs*.—These common animals have long been used in exhibiting that instructive and classic experiment of all physiology courses, the circulation of the blood. Frogs for this purpose have been kept quiet in various ways, including anesthesia with chloroform, pithing, injection of curare, wrapping the animal in moist cloth, binding it on a frog-plate or on a thin board, the webs being stretched by pinning out or by cords looped on the toes, and by other more or less successful methods which usually either finally kill the frog or cause it constant suffering. The chloretone method is an improvement on all of the above. It is reasonably rapid in its action, requires little apparatus, interferes less than any other with the normal circulation, gives an absolutely quiet frog for study, and the frog usually recovers completely from the anesthetic and may be used repeatedly.

To anesthetize a frog with chloretone take about 1 c. c. or 25 to 30 drops of a 1 per cent. solution in a small, smooth-tipped pipette with a rubber bulb, *i. e.*, a straight medicine dropper, or a fountain pen filler, and inject the solution into the animal's stomach by inserting the point of the pipette into the animal's mouth and pushing it well down into its esophagus. Press slowly and steadily on the pipette bulb and force the liquid into the frog's stomach. The only difficulty is in holding the frog and opening its mouth; but this is an easy matter if performed as follows: Grasp the frog's hind legs with your right hand, place the frog's back across the palm of your left hand, with its head projecting above your thumb and first finger, and your little finger holding the frog's hind legs, when your hand is closed over the animal. A slight pull on the lower jaw is sufficient to open the mouth, which may be held open with the first finger, or with a gag made of a match pushed well back between the open jaws and extending beyond the jaws on either side. The point of the pipette should be carried well down into the esophagus so as to insure the entrance of the anesthetic into the frog's stomach. Medium and large frogs require a second and third dose of 1 c. c. each, which may be given at intervals of three to five minutes after the first dose.

The frog becomes as perfectly passive as it would be if it were dead. Careful studies of the eyes, nostrils, ears, mouth, tongue, eustachian tubes, esophagus, glottis and other parts are easily made, or the frog may be mounted on the microscope for the study of the circulation of the blood in the web of the foot. This is best done by taking a plate of glass, about four by five inches in size, and placing it on the stage of the microscope, where it is held in place by the stage clips. The anesthetized frog is placed in its normal sitting posture on the glass plate a little to one side on the objective and one hind foot is pulled far

enough away from the body to permit the webs to be spread by separating the toes as widely as possible without tearing the webs. The bottom of the frog's foot adheres to the glass and keeps the webs stretched without the necessity of tying or pinning. By moving the glass plate any one of the webs may be brought under the objective. In using low power objectives the web needs no cover, but for objectives of short working distance a bit of cover-glass, or a thin scale of clear mica, should be placed on the web to protect the objective.

It is sometimes desirable to reduce the rate of the flow of the blood in the capillaries or to reverse its direction. To check the flow, press upon the thigh with a small cylinder, *e. g.*, the handle of a dissecting needle. To reverse the current in the capillaries, roll the cylinder along the thigh toward the foot while pressure is maintained.

If the frog is kept under observation until its webs and body become dry, the web should be moistened with a drop of water and a moist cloth spread over the frog's body. To revive the frog after completing the study, place it in a loosely covered tray or jar in shallow water, which will keep the animal's legs and ventral side moist, but will not cover its mouth and nostrils.

*Tadpoles.*—When these animals can be obtained, they are useful for studies of the circulation of the blood and the morphological changes which occur during metamorphosis. They are easily anesthetized by being placed in water five parts, 1 per cent. chloretone one part, and they become passive in from three to five minutes. To mount them for the study of the circulation of the blood with the microscope, they are placed either in a watch glass with a small amount of the anesthetizing solution, or on a glass plate in a pool of the solution. To study the tadpole's mouth, turn it on its back in a watch glass. If small and reasonably transparent specimens are selected and direct sunlight is reflected by the mirror through a diaphragm of smaller diameter than the width of the tadpole's body, it is possible to observe the pulsations of its heart.

Large tadpoles are the best animals in which to demonstrate the circulation of the blood by projection on a screen without a compound microscope. For this purpose a "quarter size" projection lens is used with a very powerful oxy-hydrogen, electric arc, or solar light. The tadpole is placed in anesthetizing solution in a zoöphyte trough, or in any glass cell with polished plate glass front. The screen should be placed at twenty-five feet, or more, from the lantern so as to obtain as large a magnification as the available light will permit. Under favorable conditions, when the animal, mounted as above described, is placed in the lantern at the position regularly occupied by a lantern slide and carefully focussed, the arteries, veins and larger capillaries of the tail may be seen and the corpuscles in the blood appear as small granules which can be seen at only a few feet from the screen. The much more satisfactory projection with the compound microscope will be described in a later article.

University of Chicago.

A. H. COLE.



## The Museum.

## VI.

## THE HALL.

The Hall of the Museum is essentially its typical expression, and in the ideal museum a treatment of this unit member should be adopted which will be consistent or congruous throughout. There seem to be just two possible but widely divergent theories for hall construction in museums,—(1) the Simple Geometric; (2) the Composite Geometric. In the former, certain relations of length and

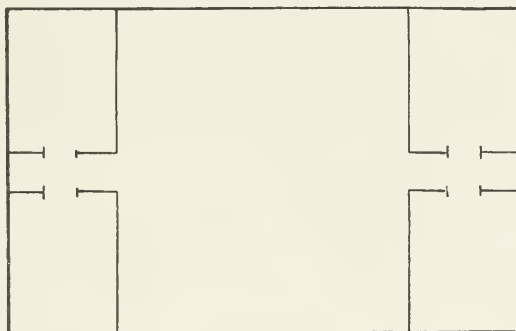


FIG. 24-a.

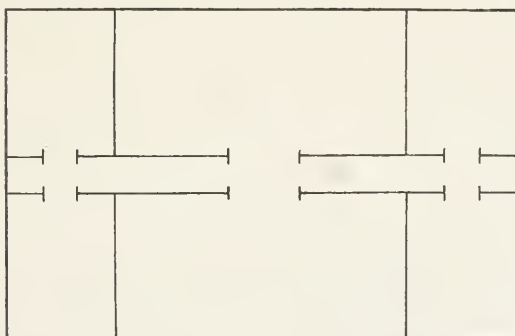


FIG. 24-b.

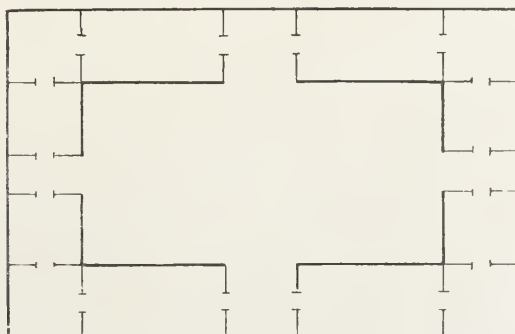
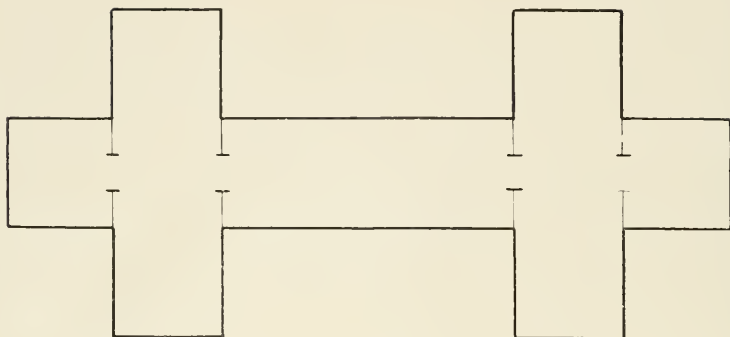
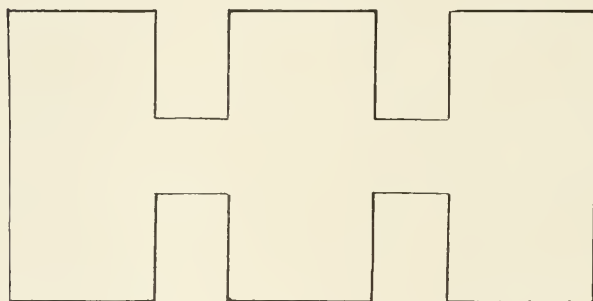
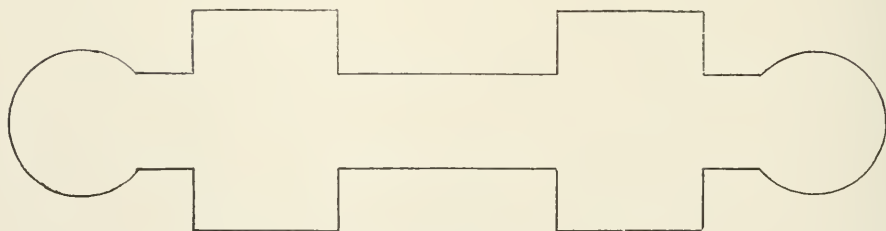
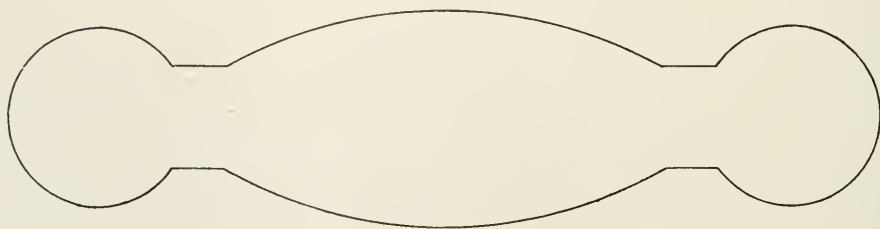


FIG. 24-c.

FIG. 24-*d*.FIG. 24-*e*FIG. 25-*a*.FIG. 25-*b*.

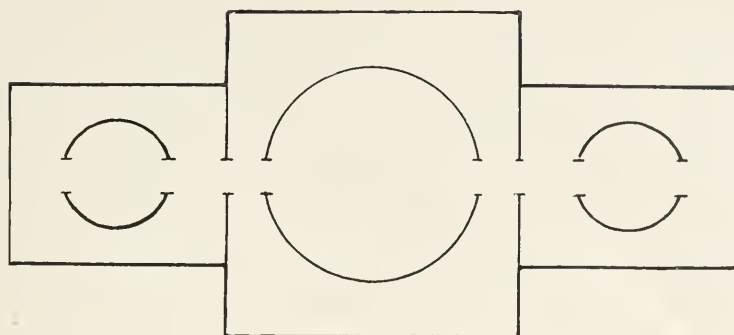


FIG. 25-c.

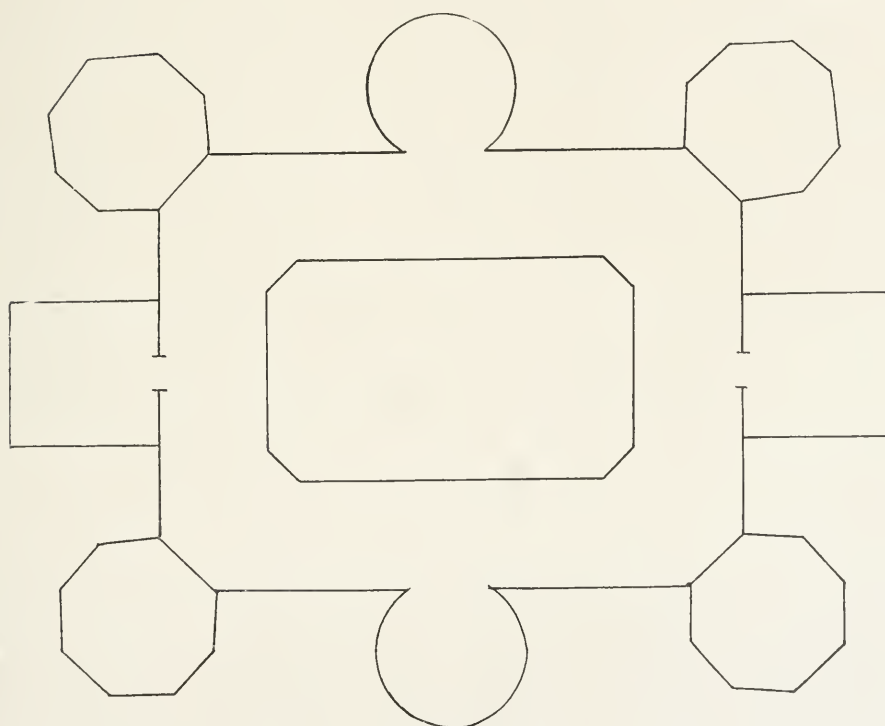


FIG. 25-d.

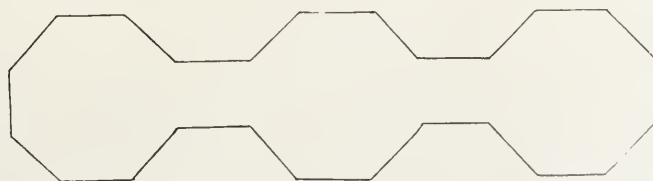


FIG. 25-e.

breadth, and rectangular or curved shapes obtain everywhere, in the latter these relations vary incessantly, and the halls are both rectangular and curved and polygonal, the combination proceeding freely throughout and determined by taste and the function subserved. There are perhaps no complete realizations of either of these theories, but it is not difficult to see that they are fundamental. The Composite, while nowhere extremely or logically developed, would perhaps

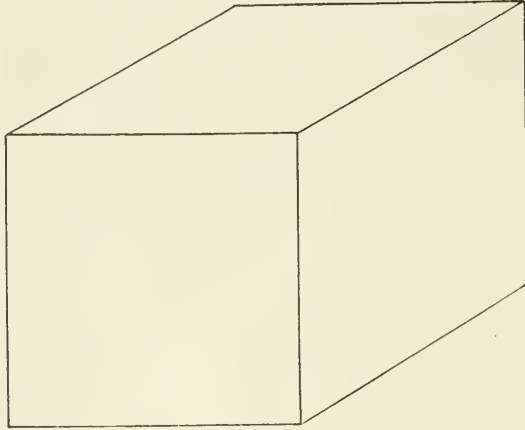


FIG. 26-a.

best describe the irregular succession of round and square, high and low halls in some Art Museums, while the Simple is equally applicable to the large museums in which the halls are square and rectangular and sensibly similar in disposition of lighting, ceiling-heights, and dimensions. Figs. 24 and 25 illustrate the plans of halls respectively Simple and Composite, and it is quite evident

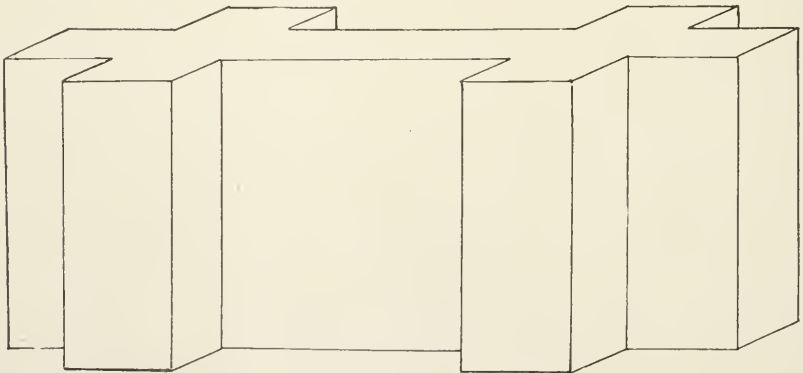


FIG. 26-b.

that in the latter group a multiplicity of variations is permissible; that in fact the vagaries of an architect can in the latter touch the limits of irresponsible creativeness. Figs. 26 and 27 show, in block inclined projections, the elevation of the plans of simple and composite halls when combined in a museum structure. In composite construction no uniformity of plan need prevail, and the



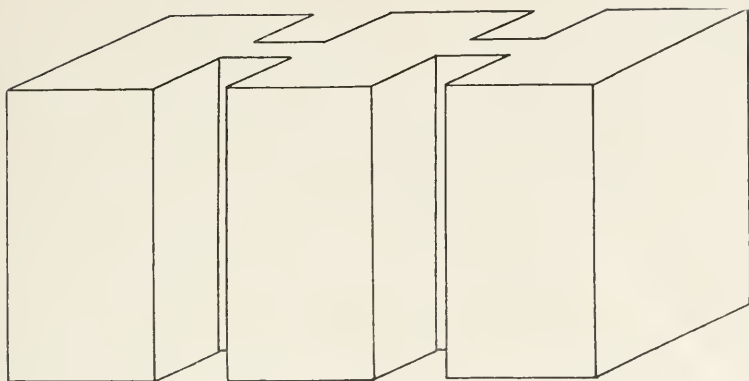


FIG. 26-c.

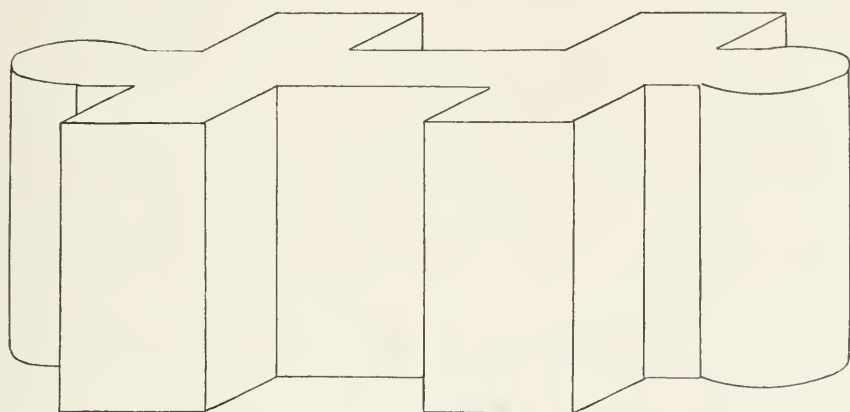


FIG. 27 a.

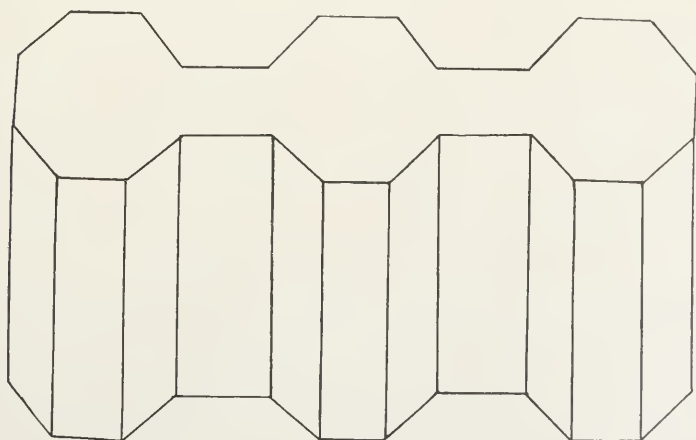


FIG. 27 b.

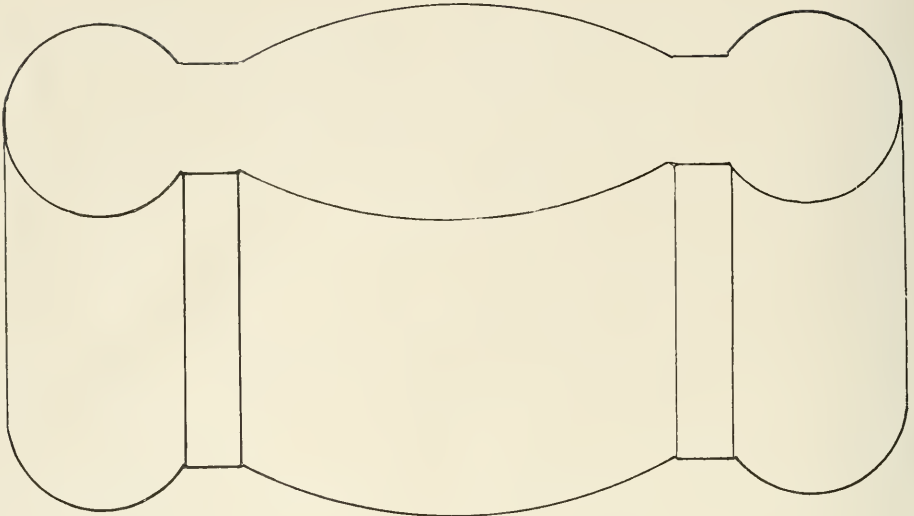


FIG. 27-c.

disposition and forms of halls may vary from story to story, a bizarrery of effect utterly confusing, expensive, and quite irreconcilable with the usual needs of museums, and only tolerable perhaps when the museum building is regarded itself as a *chef d'œuvre* of ingenious designing intended to emulate the perplexing and sometimes fascinating complexity of old cabinets, where drawers and recesses, cupboards and successional boxes pique the curiosity, and evince the skill of the cabinet maker.

The dimensions of halls admit naturally of every possible variation, but certain results seem reached by experiment, and may be summarized. Museum halls of the simple pattern should not be less than 30 feet in width, if oblong, if square not less than 50 to 60 feet. In oblong halls the longer dimension should not exceed three times the shorter; if it does, elongated and narrowing effects

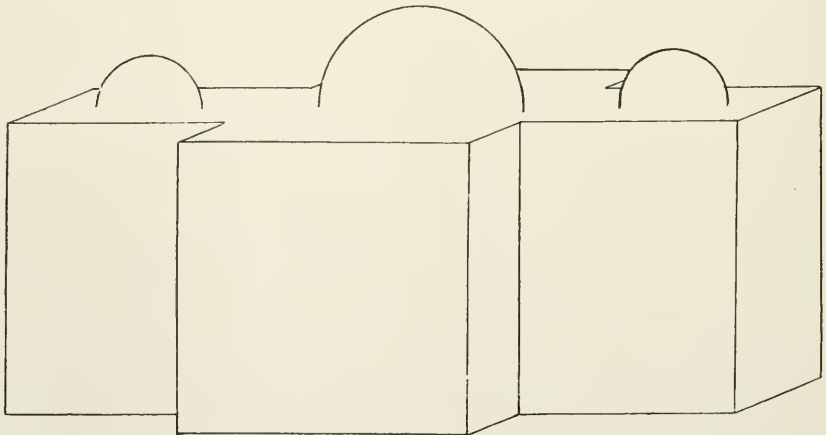


FIG. 27-d.

are produced, unless indeed in exposition buildings the dimensions are all so enormous as to preclude any possibility of dwarfing. If it is necessary to exceed this limit, viz., three times the width, in length, then the broader halls of course furnish the best results. In museums where segregation and isolation are aimed at, and subdivision is carried so far that the corals, the crustacea, the star-fish, etc., are given separate installations, much smaller halls may be used, but views

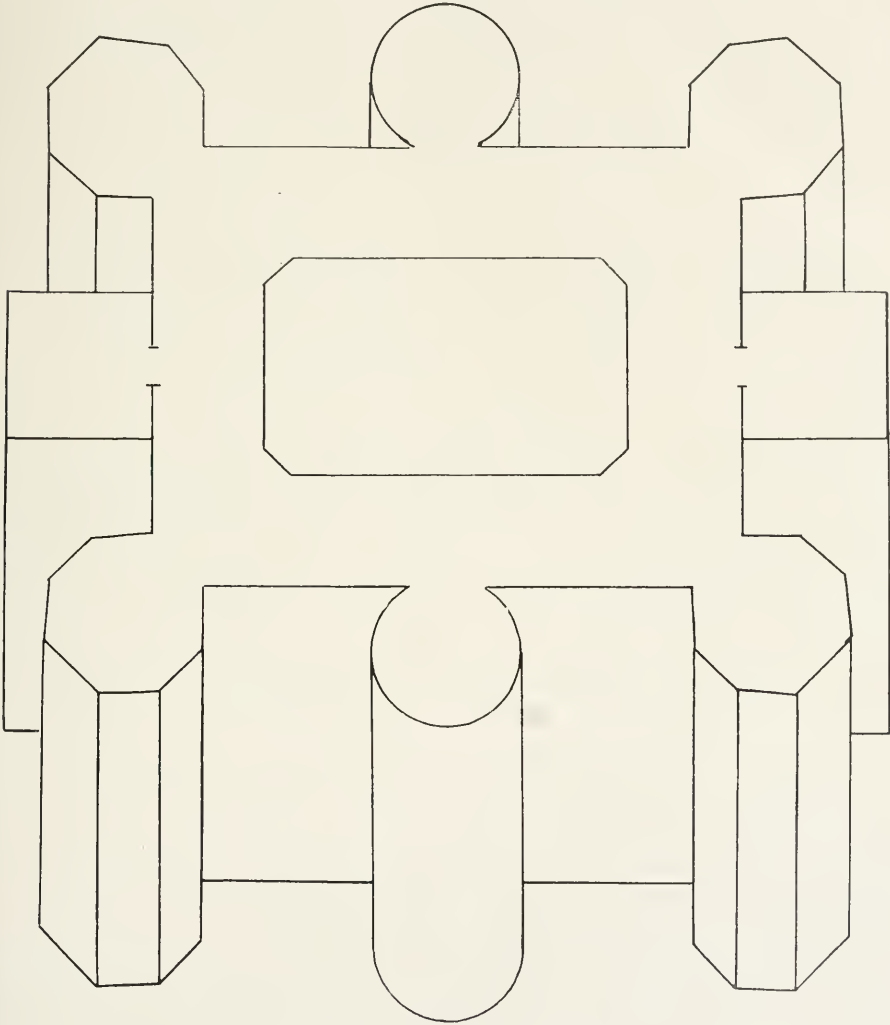


FIG. 27 c.

on that theory of construction have been sufficiently emphasized in other sections of this article. There is in Art Museums a quite necessary partition of exhibits of the same general character, where the work of a period, or a factory, of one artist or of one designer are kept apart, and a kind of chamber effect is produced by small rooms devoted to these subjects. Indeed, in art a concentration of interest and attention is secured by the isolation of masterpieces, but it

may be fairly questioned if other means of division than small halls or rooms should not be chosen as screens, temporary alcove walls, and accentuated mountings around the object, than to checker-board a great museum with cells, pock-ets, lanes and boxes.

Dimensions in composite halls may assume many degrees, but it is well, under ordinary circumstances to make the sides of a polygonal hall *not less* than 15 feet, which allows for windows; the diameter of circular halls *not less* than 30 feet if terminal, or *not less* than 45 feet if central, and the longer axis of elliptical halls *not less* than 100 feet. Such directions are suggestive and afford a table of reference from which variations may be reasonably made.

American Museum of Natural History.

L. P. GRATACAP.

## Laboratory Outlines for the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

### THE HIGHER FUNGI AND LICHENS—Continued.

XXXVI. *Puccinia graminis* Pers. Wheat Rust.

Class, Hemibasidii. Order, Uredinales. Family, Pucciniaceæ.

The æcidium stage of the wheat rust occurs in the spring on the leaves of *Berberis vulgaris*; the uredo stage, known as red rust, and the teleuto stage, known as black rust, occur on the wheat plant. The infected leaves of the barberry may be preserved in 70 per cent. alcohol and the wheat leaves and stems may be dried or also preserved in alcohol.

Æcidium stage.

1. Study the under side of a barberry leaf containing the rust under dissecting microscope. Sketch an entire leaf, representing the position of the diseased spots.

2. Under low power draw a spot showing the æcidia—cup-like bodies containing the æcidiospores.

3. Under low power examine a spot on the upper side of the leaf and note the little crater-like openings, which are the necks of sac-like bodies, called "spermogonia" (pycnidia), containing thread-like conidia. Draw.

4. By means of elder pith or strips of carrot and a razor cut cross sections of the leaf, mount, and study under low power. Under high power draw an æcidium, showing the æcidiospores. How are they developed. Draw a spermogonium with conidia.

Uredo stage.

5. Under low power, study the diseased spots on the leaves and stems of wheat (*Triticum vulgare*). Draw a patch, showing how the spores break through the epidermis.

6. Pick out some uredospores with a needle, or if fresh material is at hand cut cross sections of the stem, mount, and draw the uredospores under high power.



Teleuto stage.

7. Under low power draw a piece of wheat stem containing the black patches of teleutospores.

8. Pick out some of the teleutospores, or cut cross sections of the stem, mount, and draw a number of spores under high power. Note that the spore is made up of two cells. Study variation in individual spores.

Basidium stage.

9. It is difficult to germinate rust spores in artificial cultures. They germinate most readily in spring when those in the field are germinating. Germinate teleutospores in a drop culture and study the development of the promycelium (basidium) bearing basidiospores. Draw and describe.

10. Describe in detail the mode of growth and life history of this rust, noting especially the presence of heterœcism.

XXXVII. (a) *Fomes applanatus* (Pers.) Wallr.

Class, Basidiomycetes. Order, Agaricales. Family, Polyporaceæ.

This fungus is common on logs and stumps, forming semi-circular brackets or shelf-like bodies from a few inches to a foot or more in width. It is of a grayish-brown or white in color.

1. Draw the entire fruiting body and describe. The vegetative mycelium is in the wood from which the fruiting body projects.

2. Under low power study a patch of the pores on the under side, by simply laying the fungus on the stage of the microscope and focusing properly. Draw.

3. From a fresh specimen cut cross sections of a piece of the pore-bearing layer, mount, and study the basidia projecting into the cavity of the pores. How many spores on each basidium. Draw a single spore.

4. Mount some of the brown, woody mycelium from the upper part of the fruiting body. Draw and describe the structure of the fungus.

5. Is the plant a parasite or a saprophyte? Notice the position of the hymenium (pore-bearing surface) in relation to the surface of the earth. Is the mycelium of the fruiting body irritable to the force of gravity? Is there any advantage in this?

(b) *Polystictus cinnabarinus* (Jacq.) Fr.

This bracket fungus is very common on dry decaying logs and branches and is easily recognized by its bright red color, especially prominent on the under side.

1. Make a sketch of the entire fruiting body.

2. Under low power draw a patch of the lower surface showing the pores. Note especially the bright red color and compare it with the red color present in many flowers, fruits and roots. How do you explain the presence of the color?

XXXVIII. *Psalliota campestris* (L.) Fr. (Agaricus). Common Meadow Mushroom. Class, Basidiomycetes. Order, Agaricales. Family, Agaricaceæ.

This edible toadstool grows in open, grassy places in fields and rich pastures. The so-called "bricks" of "spawn" can be obtained from seedmen and

will keep for several years when in a dry condition. It can be cultivated by making beds of the proper character in a warm cellar or greenhouse, or in the open air in gardens. The fruiting bodies may be preserved in 70 per cent. alcohol.

1. Take some of the white filaments or bands from the ground in which the fungus is growing or from a brick of spawn, tease it out with needles and mount in water. Examine under low and high power. Note the numerous hyphæ of the mycelium and draw. This is part of the vegetative mycelium which takes up the nourishment from decaying substances in the soil.

2. Examine "button mushrooms" of various sizes and make a series of naked eye sketches showing how the button develops into the mature fruiting body or toadstool.

3. Study and sketch the mature fruiting body, showing the cap or pileus with gills on the under side, and the stalk with the annulus. Note the irregular fringe at the margin of the pileus.

4. Find the origin of the annulus and the fringe at the margin of the pileus by studying the veil or vellum of a fruiting body in which the pileus is just beginning to expand.

5. Cut off the pileus of a mature fruiting body and place it gills downward on a piece of white paper. In this way a spore print may be obtained in a few hours. Sketch the spore print.

6. Mount some of the spores and draw under high power. Color and shape?

7. Carefully cut cross sections of the gills of a pileus in which the spores are not quite mature. Mount and study under high power. Draw a part of the hymenial layer (spore-bearing layer), showing the paraphyses and the larger basidia, each of which bears two spores.

Ohio State University.

JOHN H. SCHAFFNER.

## A Paraffine Bath Heated by Electricity.\*

Under the stimulus of disastrous explosions of gas in other museums and some unpleasant experiences in our own, it was decided somewhat over a year ago to replace, as far as practicable, heating by gas in the museum laboratories with heating by electricity. The greatest danger from the use of gas is incurred where two or more lights are kept constantly burning in the same room. All of our constant burners for heating water-baths, warm ovens, etc., have been for many years supplied with the Koch automatic cut off, so that in case of accidental extinguishing of the flame, the cooling down of the burner would automatically shut off the gas supply. But the lever of the Koch burner will not always work, even though loaded with a weight greater than that which it carries when it comes from the maker; moreover, the metal on the expansion and contraction of which the tripping apparatus depends, after a time loses to a

\* E. L. Mark, director of the zoölogical laboratory of the Museum of Comparative Zoölogy, Harvard College.—*The American Naturalist*, Vol. xxxvii, No. 434.

certain extent its expansive properties, so that it fails to release the lever and cut off the gas.

The departments of Botany and Zoölogy were already in possession of a number of copper water-baths for heating paraffine and warm ovens of various constructions, which it was desirable to obtain if they could be provided with a suitable electric heating apparatus. With the aid of suggestions from Professor Sabine of the department of Physics and the coöperation of constructors of electrical apparatus, a plan was finally worked out which answers fairly well the requirements of the ordinary paraffine water-baths.

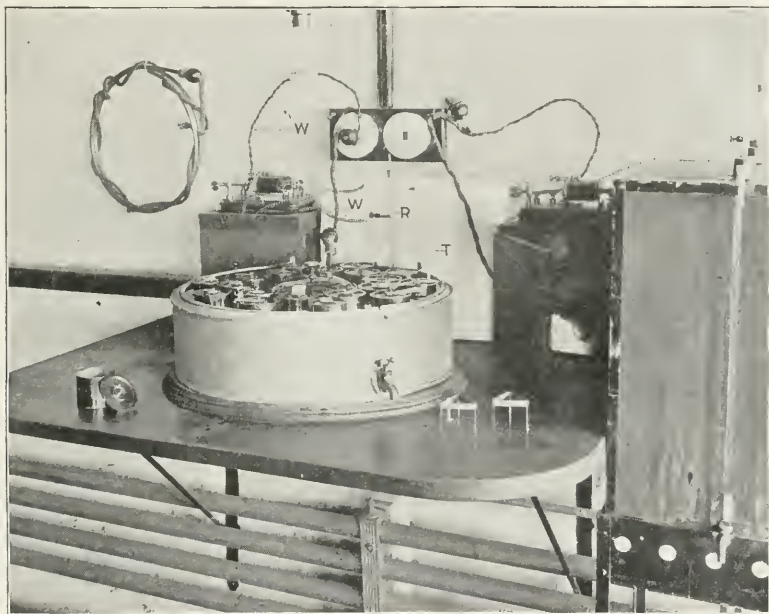


FIG. 1.—Paraffine water-bath and automatic electric heating apparatus. The 150-ohm telegraphic relay apparatus is mounted on the top of box containing a one-cell battery. Plug in place in the receptacle. At the right the plug of another heating apparatus removed from receptacle and hung on a hook. At the left a detached heating coil and insulated wire cable hanging on the wall. *R.*, regulator; *T.*, thermometer; *W.*, wires of heating circuit; *W'*, wires of relay circuit.

There were two chief problems to be solved: first, to ascertain the minimum heating capacity of the heating coil necessary for each bath; secondly, to devise an automatic regulator to control the electric heating current. To reduce the amount of heat lost, each bath was provided with a felt jacket,—covering all parts except the top,—enclosed in a canvas cover. The felt was about half an inch thick, and the canvas cover, after being slipped over the felt, was laced up on one side, as is shown above the faucet in Fig. 1. The manufacturers of the heating apparatus employed (the Simplex Electric Co. of Boston and Cambridge) then determined empirically the proper resistance and length of coil required to maintain a given temperature in each of the different forms and sizes of baths. To insure uniform distribution of heat, the coil should make at

least one whole turn. As constructed for our paraffine baths the coil is a somewhat flexible cylinder, about half an inch in diameter, with copper covering. With its attached insulated wires such a coil is shown hanging on the wall at the left in Fig. 1. The resistance metal used in the coil was a copper-nickel alloy. In the case of the paraffine baths this coil was simply shoved into the bath through an inch hole made in the top of the bath. The knob with connecting wires protrudes outside, and the coil rests on the floor of the bath, immersed, of course, in water. In the case of the thermostats used for incubating purposes, etc., a hole was cut in the side or bottom of the water reservoir, and the coil, after being introduced, was soldered in place so as to close the hole.

To devise a regulator was more difficult. The use of an ordinary rheostat proved to be impracticable, because it was not possible to make sufficiently fine

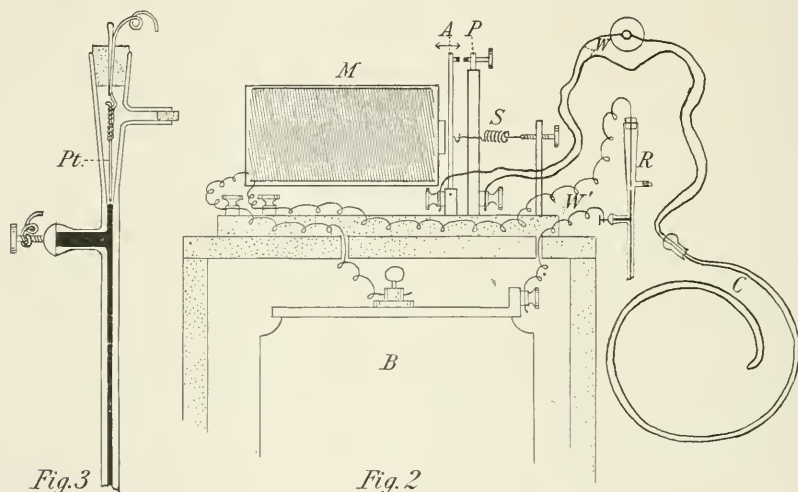


FIG. 2.—Diagram to show the connections of heating coil and circuit with relay battery, electro-magnet and mercurial regulator. *A.*, armature; *B.*, battery; *C.*, heating coil; *M.*, electro-magnet; *P.*, post in the heating circuit; *R.*, regulator; *S.*, spring; *W.*, wire of the heating circuit; *W'*, wire of the relay circuit.

FIG. 3.—Upper part of mercurial regulator—Reichert's gas regulator modified—drawn to larger scale than Fig. 2. *Pt.*, platinum wire.

gradations of resistance for different temperatures, nor by it to provide against fluctuations in the initial current.

The method finally adopted utilizes a one-cell relay battery to magnetize an electro-magnet. The heating current is made to pass through the armature, which is pulled into contact with a vertical post by means of a delicate spring. When the armature is drawn away from the post by the magnet the heating current is broken. Into the current of the relay battery is put the regulator; when the relay circuit is closed at the regulator, the magnet operates on the armature and breaks the heating current; when the relay circuit is broken at the regulator, the armature is drawn back by the spring to its first position, thus closing the heating circuit.

Our Reichert gas regulators (*R*), already in use for the purpose of regulating



the *gas* supply to the burners, were adapted by very slight changes to the new requirements. The tubular glass stopper carrying the gas inlet was removed; in its place was put a cork, bored to receive one of the copper wires (*W'*) from the single-cell battery of the Laclanche type (Samson cell No. 2). The copper wire terminates with No. 20 platinum wire (Fig. 3, *Pt.*) which is about an inch long; the height of the lower end of this wire above the mercury in the column can be roughly adjusted by moving the copper wire through the cork and wedging it in place by a small wooden wedge occupying with the wire the hole in the cork. The contact of the mercury with the platinum wire serves to close the circuit of the battery, and the other wire from the battery being connected to the mercury by means of the screw and piston in the side tube used to adjust the height of the mercury column. Into this single-cell circuit is put a standard 150-ohm telegraphic relay machine of the pattern used by the Western Union Telegraph Company.

The closure of this single-cell circuit magnetizes the core of the electro-magnet (*M.*), which, pulling against the delicate spring (*S.*), overcomes it and moves the armature (*A.*) away from the post (*P.*), thereby *breaking* the heating current.

When, owing to the interruption of the heating current, the bath cools, the mercury in the regulator recedes from the platinum point, thus breaking the *relay* circuit, the electro-magnet becomes demagnetized, and the delicate spring pulls the armature into contact with the post and thus closes the *heating* current. The finer adjustment of the distance between platinum point and mercury in the regulator is effected, as in gas regulation, by the screw and piston working on the mercury in the horizontal arm of the regulator.

The points on armature and post at which the heating current (110 volts alternating) is made or broken require to be made of heavy platinum wire (No. 15), for otherwise there is danger that the metal will fuse.

The paraffine baths of the form shown in Fig. 1 are about nineteen inches in diameter and five inches deep. The current required to heat such a bath is approximately equal to that of four 16-candle-power lamps. This could be much reduced by enclosing the bath in a glazed frame, one side of which would have to be movable to permit access to the cups.

The whole apparatus, except the heating coil, was furnished and installed by Clark & Mills, 23 Church street, Cambridge, and 543 Boylston street, Boston. It cost, including the heating coils, between \$25 and \$30 for each bath.

In determining the resistance to be used in the heating coil, one should make it as low as possible consistent with the maximum heating capacity required; for with greater heating capacity the heating current will be in operation a shorter time than with less heating capacity, and consequently the *relay* current—which is in operation whenever the heating current is not—will be required to work longer, and therefore the battery will become exhausted sooner. When the battery is so far exhausted that it will no longer magnetize the core sufficiently to overcome the spring and break the current, the regulation fails and too high a temperature results. However, a single cell will, with proper care, last for several months without renewal. To guard against the danger of too weak a relay current, one should test the current from time to time with a voltmeter.

There is one difficulty with this mercurial regulator, due to the oxidation of the mercury at the time of making and breaking the relay current. This, in time, causes a deposit on the platinum wire of oxide, which acts as an insulator and thus prevents a sharp contact. The deposit may be removed, however, by immersing the platinum wire from time to time in weak nitric acid, and if a layer of high-test kerosene oil covers the mercury in the regulator, the oxidation may be prevented.

## LABORATORY PHOTOGRAPHY.

L. B. ELLIOTT.

Devoted to Methods and Apparatus for Converting an Object into an Illustration.

In many of the processes of developments in the laboratory stained negatives are likely to result and, as they are usually considered a hopeless blemish to the negative, we believe the following paper on the "Removal of Developer Stains," by R. E. Blake-Smith, contains many practical suggestions for the removal of stains from negatives, and which we take in part from "Photography," will be of practical value to our readers:

"In most text-books of photography 'clearing solutions' are described which many of the writers affirm remove the yellow or brown stains caused by a carelessly used developer. These solutions are merely acid solutions containing a hardening agent like alum, which prevents the acid frilling the film. The best of these solutions is probably a three per cent. solution of hydrochloric acid, which is applied to the plate after it has been soaked in the formalin bath.

"These solutions may be safely relied upon in the majority of cases to lighten the stain, but they will never remove it altogether.

"If one needs a more radical cure, as will be necessary in the case of uneven stains, one must adopt much the same process as is applied in 'bleaching' fabrics. The two chief bleaching agents of commerce are bleaching powder and sulphurous acid. The former, which is by far the most important, is manufactured by passing chlorine over slaked lime. Bleaching powder is used in acid solution, and bleaching by oxidation, owing to the action of chlorine and hypochlorous acid. Sulphurous acid bleaches by reduction, and its use is limited.

"My present object is to bring before photographers the use of these two substances as applied to the removal of developer stains. First, however, it must be said that the nature of such stains on a negative varies very much with the composition of the developer and with the way in which the stains themselves have been formed.

"One thing is certain; it is always better and quite easy to take precautions against getting any developer stains on one's negatives. Plenty of a soluble sulphite should be used in the developer itself, and after development the plate should be washed at first in a solution of sodium sulphite, or directly fixed in the bisulphite hypo bath—the 'acid' bath. When the time comes for drying the negative it should be put up to dry in some place where there is no chance of it being splashed with injurious solutions. Still, owing to an occasional lapse into careless ways, one may get every now and then an unevenly stained negative.

"Then, the first process which should be gone through is to harden the film of the negative in the formalin hardening bath. It can after that be treated with

Sodium sulphite, . . . . .	35 grains.
Sulphuric acid (concentrated), . . . . .	12 minims.
Water, . . . . .	1 ounce.

“ This solution does not injure the silver image or the gelatine film in any way. It will entirely remove a few stains, especially if applied before the stain has time to dry in thoroughly, and will lighten the color even of those produced by hydro-quinone to a considerable extent. But, as a rule, it will be found that such a solution as

Bleaching powder, . . . . .	30 grains,
Concentrated hydrochloric acid, . . . . .	40 minims,
Water, to . . . . .	5 ounces,

is necessary for complete stain elimination.

“ It is important when making up the bleaching powder solution to see that no particles are left undissolved. I always dissolve the bleaching powder first in the water, and, after stirring it about, filter; then I add the acid. I believe that the above solution is capable of bleaching out entirely any stain which could possibly be formed by any developer, if sufficient time be given. It will bleach the stain images produced by pyro and hydro-quinone due to too little sulphite.

“ The weak point is, of course, that it attacks the silver image, forming silver chloride, and on account of this property, I have found it a very excellent solution for indirect sulphuretting purposes. The obvious way of dealing with the bleached negative when it has been quite freed from stain is to redevelop the image with any suitable developer, which, since the image is of silver chloride, must not contain much sulphite. Great care must be taken to wash the negative very thoroughly between the bleaching powder solution and development, as otherwise, when using a phenolic developer, a brown stain is produced all over the negative, owing to the oxidation of the phenol by the last traces of the bleaching solution. However, this method unfortunately restores most stains to some extent. Ferrous oxalate appears to do so least. It is better to treat the plate with a sulphuretting agent, such as a solution of sodium sulphite or sulphuretted hydrogen; of course, washing between the bleaching and sulphuretting solutions. But even this method appears to restore some kinds of stains to a small extent. Perhaps, without blackening, the negative could be backed with black paper and then photographed; or, if it had been very considerably intensified with silver (mind, silver—not mercury) before the application of the bleaching powder bath, it might be printed from as it is, that is, with a silver chloride image, but I have not tried experiments on these lines.

“ Very similar to the acid bleaching powder solution are solutions of chlorine and bromine, or an acidified solution of a hypobromite, but iodine solution is not so efficient as a bleaching solution for stains as the above.

“ Alkaline hypochlorite and hypobromite solutions will bleach a stain completely if allowed to act for sufficient time. When the stain is of such a nature as to be bleached quickly, these solutions can be so mixed as not to attack the silver image to any appreciable extent. The slight amount of silver chloride or bromide formed (that is, if any at all is formed) can be removed by hypo solution without any restoration of the stain. The following is the best solution of the kind I have tried:

Bleaching powder, . . . . .	47 grains.
Caustic soda, . . . . .	8 grains.
Water, to . . . . .	9 ounces.

"The solution is stirred until all the soda has dissolved, and then filtered. This bath should not be kept long, at all events when exposed to the carbon dioxide of the air. When the exposure of a negative to such a solution has to be prolonged, the solution itself should be changed about every half hour or forty minutes. But when the action of the alkaline bath on the stain is slow, one may as well use the acid bath, as prolonged exposure to the alkaline bath causes the image to be very considerably, if not entirely, chlorinated. The action goes on to a greater extent when the amount of alkali in the bath is lessened, as happens when carbonic acid is absorbed from the air. The advantages of the acid bath, when we cannot help chlorination, are that it does not apparently harm the film, and it is rather more powerful in its bleaching properties.

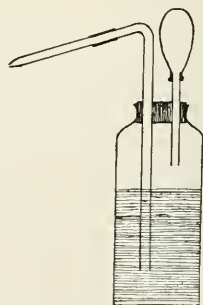
"It is a most unfortunate fact that these alkaline hypochlorite and hypobromite solutions affect the gelatine film most objectionably, causing frilling of an extreme character, and if too strong, dissolving it together. The solution above given has these faults, I believe, in a minimum degree.

"Alkaline hypochlorite and hypobromite solutions have a pleasant seaweed like smell. This is due to free hypobromous or hypochlorous acid, which is formed by hydrolysis even in the presence of large excess of alkali, and is really the active part of the solution.

"Summing up, I may say I believe that all developer stains can be bleached out by the use of an acid or alkaline bleaching powder solution, although it may not always be possible entirely to prevent the recrudescence of the trouble when the negative has to be reblackedened, owing to the image having been chlorinated."

### A Microscopical Reagent Bottle.

The accompanying sketch shows a form of dropping bottle for containing and applying microscopical stains and reagents, which has been found useful in histological work. It is easily fitted up, if a small wide-mouthed bottle provided with a tight-fitting cork be procured. A couple of small pieces of glass tubing, an india-rubber bulb, and a tiny piece of india-rubber tubing to connect up the outlet tube will complete the arrangement. Briefly, its advantages consist in keeping the reagent free from contamination with dust, and allowing of its removal without taking out a stopper or cork. In addition, complete control over the amount of reagent deposited on a slide is obtained by varying the hand-pressure on the bulb. An empty bottle of this description can also be utilized to remove excess of liquid from a slide, by pressing the bulb tightly, inserting the outlet tube in the liquid, and then gradually removing the pressure on bulb, when of course the liquid will be forced up the tube by atmospheric pressure, as with an ordinary syringe. A wide-mouthed test tube, if fitted up like this, will be found useful in picking out the larger forms of pond life from a large gathering, by using it in a similar way to a pipette.—*Eng. Mech. and Eng.*, No. 1984.





## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

**Schumann, K.** Ueber die weiblichen Blüten der Coniferen. Verhandlungen des Botanischen Vereins der Provinz Brandenburg, 44: 5-80, 1902.

Prof. Schumann has made an extensive comparative study of the ovulate flowers of the Coniferales (including *Ginkgo*).

Little attention is given to the earlier

developmental stages, but the later stages and the mature flowers are described in great detail and many suggestive comparisons are made. Teratology is not regarded as of much value in "explaining" morphological relations or establishing homologies.

The more important conclusions are: that the Cupresseæ are related to the Taxodiæ and that of living forms the genus *Sequoia* is nearest the point of contact. This does not mean that *Sequoia* is the starting point for the Cupresseæ, but that this form must have been similar and related to *Sequoia*. This supports Potonie's theory that the Taxodiæ appeared before the Cupresseæ.

C. J. C.

**Denke, P.** Sporenentwicklung bei Selaginella. Beihefte zum Bot. Centralbl. 12: 182-199, pl. 5, 1902.

Since the difficulty in securing good preparations has hindered accurate work on the finer structures of Selaginella,

any suggestions as to methods of treatment are welcome. The usual Flemming's solution and also that of Hof causes a collapse of protoplasmic structures, and material so fixed does not stain well. Cones treated with boiling water for 4 to 5 minutes and then gradually dehydrated in alcohol also proved unsatisfactory. After trying these and several other fixing agents, Dr. Denke recommends the following method:

Absolute alcohol	-	-	-	-	2 parts.
Glacial acetic acid	-	-	-	-	1 part.

This solution is allowed to act for 26 hours, after which the material is placed in 50 per cent. and then in 70 per cent. alcohol, 2 hours each; 70 per cent, 12 hours; 80 per cent. and 95 per cent., 24 hours each; absolute alcohol, 7 hours; clear in chloroform (10 to 24 hours?); in paraffin bath at least 8 days. Sections were cut 5 to 7.5 $\mu$  thick and stained in Haidenhain's iron alum hæmatoxylin with or without a counterstaining with Congo red. Clove oil was used for clearing.

The following are some of Dr. Denke's conclusions: The cauline sporangia originate from epidermal cells and underlying tissue. In the microsporangium a considerable number of spore mother-cells fail to undergo the tetrad division. Such cells lose protoplasmic contents and function like tapetal cells. Microsporangia and megasporangia develop alike up to the spore mother-cell stage, and in phylogeny probably represent a common structure. In both microspore

and megaspore mother-cells the spindle is extra-nuclear in origin, and it appears while the nucleus is still in the resting stage. The spindle soon becomes bipolar and in its behavior recalls the spindle in the spermatogonia of the salamander. The formation of the membranes of the four young spores is peculiar, and, in spite of the attention which has been given to the subject, a conclusive account has not yet appeared. From the cytoplasm of the young spore, two membranes are formed one after the other. At first they grow in contact with each other, but the outer membrane, by a more rapid growth, becomes separated from the other and a space appears between them. The inner—the mesospore—does not arise by a mere splitting of the outer layer, or exospore, as some have supposed. The delicate wall formed around each of the four young spores at the division of the spore mother-cell is dissolved.

C. J. C.

**Möbius, M.** Botanisch-mikroskopisches Praktikum für Anfänger. 8vo, pp. ix + 121. Gebrüder Bornträger. Berlin, 1903.

This book contains eighteen exercises, each intended to occupy about three hours. Directions are given for mak-

ing 65 preparations, 42 of which are Phanerogams and 25 Cryptogams. Almost without exception, the methods are those employed for examining fresh material, no microtome being used and almost no attention being given to embedding, staining, etc. In this country, subject matter like that contained in this book is usually presented by the teacher in the laboratory.

C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

**Holmgren, Nils.** Studien ueber Cuticularbildungen. Anat. Anz., **22**: 14-20, 1902.

The author has already shown in a previous paper (review in this JOURNAL

for Dec., 1902) that the striated border in certain cells is only a transformed ciliary surface. He continues his studies on the mouth-shield skin and mid-gut epithelium of *Chaetoderma nitidulum*. All material was fixed in Perenyi's, Flemming's, or sublimate solutions, and stained in iron-hematoxylin with congo-red. The mouth cuticle shows a thin cuticle overlying a wide striated border; beneath there is a layer of nearly cubical cells with a very perfect and distinct row of blepharoblasts along their outer margins. The author shows this striated border to be the cuticularized cilia. The thin outer cuticle is believed to be a true secretion product. The cuticle of the body skin is for the most part structureless and seems clearly a product of the matrix cells. In certain parts of the hypodermis there are, however, anomalous cells and cuticle formation. In these, which occur more numerously at the anterior end of the animal, can be seen in iron-hematoxylin preparation an outer layer of deeply stained granules, from

which passes out into the cuticle a bunch of cuticularized fibers. These are clearly cilia, arising from blepharoblasts. In the mid-gut are quite frequently ciliated cells, although the number of cilia per cell is greatly reduced, most cells have but few, many only two, in all parts of the mid-gut. The epithelium is covered with a thin, vertically striated cuticle, through which the few cilia project. Further, each cilia has its blepharoblast, and in the cells where only two cilia exist, two bodies are formed, resembling in position and appearance the cell centrosomes.

A. M. C.

**Webb, T. C.** Apparatus for Removing Pieces of Tissue for Microscopical Examination. Journ. Brit. Dental Ass., **23**: 438-440, 1902.

This apparatus is designed to remove tissue by suction. A piece of glass tubing, three-eighths of an inch in diameter, is attached to an aspirator, the tissue is drawn up into the tube and easily snipped off with the scissors.

A. M. C.

**Werner, R.** Artificially Induced Anomalies in Cell Division. Archiv. Mikr. Anat., **61**: 85-122, pl. 1, 1902. Rev. Journ. Roy. Micr. Soc., Pt. 6, 365, 1902.

The author experimented with the effects of ether spray. The cold brings about proliferation and cell-lesions, abnormal modes of cell division ensue.

Most are amitotic, some are mitotic. The amitotic are in a sense simplified mitosis; there is an unsymmetrical and incomplete rearrangement of the chromatin before metakinesis, and on this other phenomena depend. Although not of equal value to mitosis, viable cells result from this amitotic process. Giant cells of unicellular origin arise either by stimulation of centers and paralysis of the periphery (Weigert) or by a hindering of cell-wall formation through persistent activity of the centers (His).

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

**Cunnington, W. A.** Studien an einer Daphnide, *Simocephalus sima*. Beiträge zur Kenntnis des Centralnervensystems und der feiner Anatomie der Daphniden. Jenais Zeitschr. N. F. Bd. **30**: 447-520, Taf. 24-26, 1903.

On account of the protecting exoskeleton it is absolutely necessary to use fixing agents which penetrate with ease and rapidity. Picric mixtures, especially the stronger picro-sulphuric of Kleinenberg, were therefore employed.

The ether-alcohol mixture employed by G. W. Müller for *Ostracoda* interfered with the subsequent staining of *Simocephalus*. The best results were obtained by using a saturated solution of picric acid in an alcohol (1 part) and ether (5 parts) mixture. The animals were shaken in this fluid for 1 minute, then transferred to 70 per cent. alcohol until the picric acid is all removed. They were then stained in toto in Ehrlich's hæmatoxylin and counterstained on the slide in orange G. In order to avoid the aqueous solution of orange G ordinarily

employed, the author added a little acetic acid to 70 per cent. alcohol, which will then dissolve sufficient orange G to stain the sections deeply in one minute.

C. A. K.

**Leger, L., et Duboscq, O.** Les Grégarines et l'épithélium intestinal chez les Tracheates. Arch. de Parasitologie, 6: 377-473, pls. 2-6, 1902.

This is a study of the process of infection by the parasite and of the means of defense by the host. Ripened sporocysts from parasitized individuals were

fed to uninfested ones and the process of attachment of the sporozoites to the intestinal epithelium and the growth of the parasite traced to the adult condition. In some cases, as in *Stylorhynchus*, the sporocysts are not mature when voided. When freed from impurities they are placed in a sterilized moist chamber on a fragment of charcoal, where they reach maturity without alteration. Thymol used by Labbè as an antiseptic with the Coccidia is not recommended by the authors for the Gregarinida. Ripe sporocysts placed in the gastric juice of their normal hosts will dehisce in a short time as a rule. With *Diplocystis*, however, dehiscence was secured only within the digestive tract of the animal. In this case it was found possible to substitute for the normal host a closely related species, *Gryllus campestris*. Artificial infection is best accomplished by keeping the animals to be infected without food or water or even moisture for some days. The ripe sporocysts are then mixed with a small quantity of food and fed to the famished animal. At desired intervals the infested insects are killed, their mid-guts dissected out and spread out in the killing fluid. Flemming's fluid, saline or acetic sublimate, and von Rath's fluid were used for fixing and hæmalum with various counter stains, iron hæmatoxylin, safranin with picric acid, light-green, or picro-indigo carmine, and Flemming's triple stain for coloration. C. A. K.

**Maier, H. N.** Ueber den feineren Bau der Wimperapparate der Infusorien. Arch. für Protistenkunde, 2: -73-379, Taf. 3, 4, 1903.

The infusoria were killed with osmic acid fumes and then treated with a 5 per cent. solution of soda, after the

method of Schewiakoff, for study of the arrangement of the cilia. The relation of the cilia to the cytoplasm was studied in sections. The larger infusoria visible to the unaided eye were picked up individually with a pipette and transferred to the killing fluid. The smaller species were secured by mixing equal quantities of the water, or infusion containing them, and of the killing fluid. The mixture was then sedimented in a centrifuge. Cold sublimate-alcohol (1 part absolute alcohol to 2 parts of a 5 per cent. solution of sublimate in 0.6 per cent. salt solution) gave the best service as a fixing agent, though strong Flemming's mixture was excellent for plasmatic structures. After fixing the animals were transferred with a pipette to a glass tube 10 to 15 mm. long and about 2 to 3 mm. in diameter, the lower end of which had been closed by a plug of fern-wool. The tube is then filled with the desired fluid and the upper end is also closed in a similar fashion. The tube and its contents are carried up through the alcohols to chloroform, chloroform-paraffin, and finally to pure melted paraffin. The upper plug is then removed to hasten the evaporation of the chloroform. After standing for some time in the paraffin the tube is removed and the paraffin allowed to cool. When cold the tube is slightly warmed in the hand and the



paraffin cylinder is then pushed out with the aid of a wooden plunger. This cylinder is then embedded in a watch-glass as follows: Hardened paraffin in a watch-glass is melted in an area large enough to receive the cylinder by means of a hot metal rod, and the cylinder containing the embedded infusoria is placed therein and the whole is cooled. Prismatic blocks can then be cut from the mass and sections cut in the usual fashion.

The larger infusoria were likewise handled in tubes through the process of infiltration, and were then poured out into a watch-glass of melted paraffin and oriented in the desired position under a dissecting microscope. Series of sections from 1 to 3 microns in thickness in any desired plane were thus secured.

Sections were fixed to the slide by the distilled water method and after preliminary coloration in a weak solution of Bordeaux red for six hours they were washed in water, mordanted for 12 hours in a 3 per cent. solution of iron alum, washed in running tap-water for 10 minutes, and stained in Heidenhain's hæmatoxylin for 24 hours. After differentiating to the desired degree in a 3 per cent. solution of iron alum under the microscope and washing for 20 minutes in running tap-water, the sections were mounted in the usual fashion. *Intra vitam* staining with a weak solution of neutral red (0.001 per cent. after the method of Putter) was employed for the demonstration of certain plasmatic structures.

C. A. K.

**Woltereck, R.** Trochophora Studien I. Ueber die Histologie der Larve und die Entstehung des Annelids bei den Polygordius-Arten der Nordsee. *Zoologica*, 13: Heft. 34, 71 pp., 11 Taf. und 25 Textfiguren, 1902.

The larvæ were fixed in Flemming's fluid, in a saturated solution of corrosive sublimate in sea-water, or by preference in Hermann's fluid or in a sub-

limate-acetic-alcohol mixture composed of equal parts of 80 per cent. alcohol and of a saturated solution of sublimate, plus sufficient acetic acid to give a 10 per cent. solution. To all these fluids a few drops of formal were added whenever it was desired to preserve the finer elements of the nervous system with greater precision. The author found the varying degrees of staining obtainable with Heidenhain's iron hæmatoxylin of great value in differentiating tissues, especially the contractile elements. Beautiful preparations of the nervous system were obtained by the use of Apathy's hæmatein I. A. This leaves the muscles colorless, but differentiates the ganglionic cells and their fibres, as well as the neurofibrillæ and the primitive plasma stains of the diffuse nervous system. This stain is capricious and must be used with reference to the killing agent, the age of the larva, etc. The right duration of the staining (about 2 to 3 days) is important, as is also the differentiation of stain in absolutely pure water for 1 to 2 days.

By means of sharp ophthalmic needles the spherical larvæ were cut in two and flat preparations made of the hemispheres. The author commends highly the stereoscopic lens for this delicate operation, as well as for the dissection, embedding, and orienting of these small objects. The clove-oil-collodion method of Hoffmann was used for embedding.

C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory,  
University of Michigan, Ann Arbor, Mich.

**Verworn, M.** Die Biogenhypothese. Eine kritisch-experimentelle Studie über Vorgänge in der lebendigen Substanz. Jena (Gustav Fischer), 1903. Pp. iv and 114. Mk. 2.50.

This book is an expansion of the theory of the fundamental physiological basis of vital phenomena first sketched in outline in the author's well known

"Allgemeine Physiologie." The theory is advanced merely as a working hypothesis, and regarded in this light it will be found extremely suggestive by all biologists. By the term "Biogen" the author designates the chemical substance in protoplasm by whose metabolism (both destructive and constructive) vital phenomena are produced. At the outstart a detailed comparison between the catalytic action of enzymes and the metabolic processes taking place in living substance is made, and the conclusion reached that the biogen molecule may be regarded as very similar to an enzyme. On the basis of various experimental facts the following composition is suggested for the biogen molecule: as the center and basis of the molecule we may have a nitrogenous compound of the character of a benzol group. With this central group are associated two side-chains; one a compound of the carbohydrate type with a terminal aldehyde group, the other a nitrogenous or iron compound. In the first of these side-chains the processes of oxidation are believed to occur, while the second acts as a receptor and translator of oxygen. With this idea as a basis the author proceeds to discuss a number of the most important physiological problems, such as metabolism, the phenomena of the action of stimuli, the source of the energy of muscles, etc. Lack of space forbids further mention of this very interesting contribution to physiological theory, which is well worth the perusal of every biologist interested in the fundamental problems of his subject.

R. P.

**Lillie, R. S.** On Differences in the Electrical Convection of Certain Free Cells and Nuclei. Amer. Jour. Physiol. 8: 273-283, 1903.

It is well known that the particles of a colloidal solution move towards and aggregate at the poles when a constant electric current is passed through the

solution. The pole to which a particular particle shall go is determined by the sign of the electric charge which that particle bears. Furthermore, the sign of the charge bears a definite relation to the chemical nature of the colloidal substance, acid particles being electrically negative and basic particles positive. In the cell the chromatin of the nucleus is strongly acid, while the cytoplasmic proteids are for the most part basic in character. On the basis of the results obtained with colloidal solutions it would then be expected that nucleus and cytoplasm differ electrically. The purpose of the present paper is to test experimentally this supposition and to point out its importance for general theories of the physiology of the cell, mitosis, etc. The experimental method used was to

determine whether the movement of cells with varying proportions of nuclear and cytoplasmic material, suspended in a fluid and subjected to the action of a constant current, was towards the anode or the kathode. The methods were as follows: "Finely divided tissues, in as fresh a condition as possible, were teased in  $\frac{2}{4}$  cane-sugar solution (isotonic with physiological salt solution) and mounted in the same medium upon a specially prepared slide, so constructed that the entire preparation while under examination could at any time be exposed to the action of the electric current. The construction of this slide is as follows: a long cover-glass (50 by 25 mm.), around which passed two tightly drawn transverse loops of thin platinum wire about 15 mm. apart, is cemented by means of Canada balsam to an ordinary microscopical slide. The platinum wires are connected through a pole changer and simple key to the poles of a battery; this has consisted usually of three storage cells with an aggregate E. M. F. of from 7 to 7.5 volts. The tissues and cells under examination are mounted in sugar solution on the slide in the space between the wires, and the behavior in the electric field can then be studied under high powers. The rate of movement is measured by means of the ocular micrometer."

It was found as a result of the experiments that some cells migrate with the positive stream, and others with the negative. Structures consisting largely of nuclear matter (e. g., sperm heads, thymus nuclei, lymphocytes) migrate with the negative stream, the rate of movement being correlated with the degree of acidity of the chromatin. Cells with voluminous cytoplasm (e. g., large leucocytes, many red blood corpuscles, involuntary muscle cells) tend to move with the positive stream. These are of course the results to be expected if the theory proposed is correct. The possible application of these results to the phenomena observed in mitotic cell division are discussed.

R. P.

**Reichert, E. T.** Quick Methods for Crystallizing Oxyhæmoglobin; Inhibitory and Acceleratory Phenomena, etc.; Changes in the Form of Crystallization. *Amer. Jour. Physiol.* 9: 97-99, 1903.

The author has found the most expeditious method of obtaining crystals of oxyhæmoglobin to be to add to the blood, either before or after laking with

ether, from 1 to 5 per cent. of ammonium oxalate. Crystallization, in the case of dog's blood, invariably begins immediately, and any quantity of crystals can be obtained within a few hours at the ordinary room temperature. Blood of the horse, rabbit, guinea-pig and *Necturus* have been found to yield crystals very readily under this ether-oxalate treatment. The author further finds that if to the blood of one species, the blood, plasma, or serum of another species be added, the laking of the blood may be retarded, accelerated, or unaffected, according to the character of the mixture. The period required for laking may be prolonged for five minutes or more. The crystallization of the oxyhæmoglobin may be hindered or prevented in such mixtures. The typical forms of the crystals of certain kinds of oxyhæmoglobin may be modified or completely changed when the bloods of two species are mixed. Thus, if to the blood of the rat there be added a definite percentage of the blood of the guinea-pig, crystals of the rat's oxyhæmoglobin may appear in unaltered form, but most, if not all, of those from the guinea-pig's blood will be changed.

R. P.

**Wagner, A.** *Vitalismus? Eine aus der modernen naturwissenschaftlichen Litteratur geschöpfte Zusammenstellung von mechanischen Erklärungsweisen für Bewegung, Stoffwechsel und Fortpflanzung der Zelle.* Berlin und Leipzig, 1902 (Vogel & Kreienbrink), pp. 57. Mk. 1.20.

**Wolff, G.** *Mechanismus und Vitalismus.* Leipzig, 1902. (Thieme), pp. 36. Mk. 1.

The first of these papers is a decidedly extensive rather than intensive discussion of the question of "mechanism versus vitalism" as the explanation of vital phenomena. The author comes to the conclusion that any vitalistic assumption is unnecessary. Biologists

will be surprised to learn from this paper that "the fact of the inheritance of acquired characters argues very strongly in favor of the correctness of Weismann's hypothesis of the continuity of the germplasm."

Wolff's paper is of an entirely different character from that above mentioned. It is a thoughtful critique, point for point, of Bütschli's pamphlet on the same subject, the present author defending the neo-vitalistic standpoint. R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt, Harvard University Medical School, Boston, Mass.

**Cushing, H.** *Physiologische und anatomische Beobachtungen über den Einfluss von Hirnkompression auf den intracranialen Kreislauf und über einige hiermit verwandte Erscheinungen.* Mittheilungen aus den Grenzgebieten der Medizin und Chirurgie, 9: 375, 1902.

The circulation of the cerebral cortex in dogs was observed by inserting a glass window in the skull. Local compression was produced by distending a rubber bag, which was attached to the inner end of a canula inserted through

a trephine opening. The degree of pressure desired was obtained by allowing mercury to run into the bag from a burette. General compression was produced by allowing normal saline solution to enter the cerebro-spinal space through a rubber tube connected with a flask. The tension was regulated by the degree of elevation of the flask.

J. H. P.

**Keyes, P.** *Lecithin as a Complement.* Berlin. klin. Woch. pp. 886, 918, 1902.

Keyes found that the erythrocytes of some animals were hemolyzed directly

by venom, while the erythrocytes of other species were hemolyzed only after a complement was added. Not all the corpuscles in any animal showed the same susceptibility. The red blood corpuscles of dog and guinea pig were most susceptible, of horse very little, and of ox, sheep and goat not at all, but hemolyze on addition of a complement.

Keyes showed that an endocomplement exists in red blood corpuscles, and that a definite chemical crystallizable substance, lecithin, can assume, in a certain sense, the role of complement. He thinks that lecithin and cobra amboceptors came into union, and by this the avidity of the cytophile group of the cobra amboceptors is heightened. He holds that the venom amboceptors, besides a cytophile group, have two haptophore groups, of which one can bind the ordinary complement and the other the lecithin.

J. H. P.



## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn, Wesleyan University, Middletown, Conn.

**Boekhoet and Ott de Vries.** Ueber die Reifung der Edamer Kase. C. f. B. ii, vii, p. 187, 1901.

ogists have believed for some time, or in large degree to enzymes, as has been claimed by Russell and Babcock. They had previously shown that it was difficult to get cheese to ripen without the presence of bacteria, and the experiments which they have now conducted have obtained two results. First, they have isolated two very important bacteria from Edam cheese which they regard as contributing largely to the ripening; secondly, they have experimented with cheese made from milk obtained under special precautions. They attempted first to obtain directly from the cow milk which was sterile, but found this an impossibility. They then adopted the plan of thoroughly washing the animals with a disinfectant solution, and drawing milk under special precautions in closed pail under conditions where the bacterial contamination was reduced to its minimum. This was done by having a long spout leading from the side of the pail and ending in an open funnel at the same distance from the closed pail. Under these conditions they obtained milk which was, not indeed sterile, but had only an exceptionally small number of bacteria. With such milk, which they called aseptic milk, they made cheeses and found that under these conditions the ripening of the cheese did not occur. Such milk of course contained all of the natural enzymes and differed only from normal milk in lacking bacteria, and they therefore reached the conclusion that, in addition to the action of galactase, the ripening of cheeses is in reality due to the action of bacteria.

**Kozai.** Weitere Beiträge zur Kenntniss der natürlichen Milch gerinnung. Zeit. f. Hyg. xxxviii, 387, 1901.

This author, a Japanese, who has previously given useful papers upon milk souring, contributes a further paper

upon the same general subject, for the purpose of verifying previous conclusions and explaining the contradictions of others. The important conclusions which he reaches are as follows: First, in spontaneous milk souring there is produced lactic acid, alcohol, acetic acid and salicylic acid. Second, the temperature at which the milk is kept has a great influence upon the kind of fermentation that takes place. In the slow processes of souring there is a more profound decomposition of albuminoids and non-nitrogenous substances than when the process goes on rapidly. In spontaneous milk souring there are three chief species of bacteria concerned. The most important of which he calls *B. acidi paralactici*, which is the same species as described by several others under different names. A second species is *B. acidi levolactici*, while a third is a micrococcus. The coli bacillus is also present and sometimes aids in the process. The second of the three species belongs to the aerogenes group and it produces left handed milk acid and a small quantity of alcohol, together with some acetic and salicylic acid. The results thus obtained are in essential agreement with those obtained by students of milk bacteriology in this country. The chief species described by Kozai is apparently identical with the *B. acidi lactici* of Esten, which is the most common cause of milk souring in America.

H. W. C.

## NEWS AND NOTES.

The Laboratory of the United States Fish Commission at Woods Hole, Mass., will be opened on June 15th for the nineteenth season of its existence. The privileges of the laboratory, including the services of the staff of collectors and use of the commission's fleet of vessels, are, as usual, extended free of charge to those competent to carry on research in marine biology. Applications for tables should be sent to the director of the laboratory, Dr. F. B. Summer, 17 Lexington Ave., New York City.

The Biological Laboratory of the Brooklyn Institute of Arts and Sciences, located at Cold Spring Harbor, Long Island, announces the summer session for 1903 to begin July 1, and continue for six weeks. Investigators may arrange to use the laboratory from the middle of June until the middle of September. Extensive courses in Zoölogy, Botany, and Microscopical Methods are arranged, supplemented by a semi-weekly Biological Club, evening lectures, and numerous excursions. For full information address Prof. Franklin W. Hooper, 502 Fulton St., Brooklyn.

The Lake Laboratory of Ohio State University, located at Sandusky, on Lake Erie, will open for the 1903 summer session on June 29th and close August 7th. The laboratory offers a number of courses in Zoölogy, Entomology, Botany and Physiology, and provides opportunity for investigators to carry on research work in any direction. Applications and correspondence should be addressed to the director, Prof. Herbert Osborn, Ohio State University, Columbus, Ohio.

The Alstead School of Natural History will hold its fifth session at Alstead Center, New Hampshire, during five weeks of the summer of 1903. The arranged courses embrace the following subjects: Ornithology—The Native Birds; Zoölogy, with special reference to Entomology; Flowers and Insects; General Botany; Trees and Shrubs; Physiography—The Making of the Landscape. Information may be secured by addressing Mr. Wm. L. W. Field, Milton, Mass.

TO MAKE A STERILE COTTON-WOOL SWAB.—Take a piece of stout copper wire about the length of a test-tube, and bend about half an inch at one end firmly over a piece of cotton-wool, twirl the wool with the fingers into a firm lump somewhat smaller in diameter than the test-tube, fix the free end of the wire into a soft cork or into another plug of wool, which closely fits the mouth of the tube, and, holding the tube within the points of a pair of Cornet forceps, pass it the full length backward and forward in a Bunsen flame for a few minutes. When the wool swab in the tube begins to show signs of scorching it is sterilized. The same result may, of course, be obtained by placing in a hot-air sterilizer. Affix a label, which is filled in when used with the name of patient, date, and clinical information.—*Jour. State Med.* XI: 3.

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## In High School Biological Work, What May be Regarded as Settled by the Discussions and Practice of the Last Quarter Century?

In the first place, we are all agreed that the very first requisite for successful biological work in the high school is the thoroughly trained teacher. This has been insisted on in season and out of season. Everybody admits it, but the fact remains that many schools in the state are attempting work of this kind with poorly prepared teachers. The remedy is to teach a less number of sciences in the smaller schools, say one physical and one biological science, and then pay a salary that will command the right kind of a teacher.

In the second place, it is a settled thing that biological instruction must be carried on largely, or even mainly, by laboratory methods. It is not long since prominent educators held very different views of this. It was thought that the biological sciences should be taught in great part as "information studies," and with reference to their supposed æsthetic or ethical value. Such a view is hardly held to-day by any scientific teacher. Those who have had really scientific training know how different this form of discipline is from that of other subjects in the curriculum, and they value the subject first of all for the peculiar discipline that it affords, a discipline not to be secured apart from laboratory instruction.

We are pretty well settled at last as to what ground ought to be covered in a high school course extending through a year. Whether botany or zoölogy is selected is of little consequence; in either subject it is agreed that morphology, physiology, and ecology should be presented so that in each of these divisions of the subject the student will at least be able to grasp the fundamental principles. That it is perfectly practicable to accomplish this is demonstrated by what is actually done at the present time in various high schools in the state. Those who believe that this is impossible would do well to visit some of these high school laboratories and see what is going on there. I wish I might add that our high school courses in biology are making provision for that "speaking acquaintance" with living things in their natural environment that is becoming

NOTE.—These papers were presented to the Michigan Academy of Science, and are intended for the use of teachers of the biological sciences in Michigan only. They are to be incorporated in a pamphlet for the use of Michigan teachers, which is to be issued under the auspices of the Michigan Academy of Science. The matter is printed here, with the hope that it may be of use to teachers working outside of Michigan.—ED.

more and more indispensable to those who would know them in their true relations. The general who knows his soldiers by name is at a great advantage over the one who knows them only as soldiers. It is very desirable that some provision should be made for getting more of such knowledge. Possibly some allowance for time spent in vacation might advantageously be given.

It is an entirely settled thing, as far as the university is concerned, how much is to be attempted. For several years it has been announced that the university would accept for admission what a properly trained teacher, working by laboratory methods, can accomplish with a class in a year. If the teacher has been specially trained in physiology and decides to emphasize that side of the subject, or is skillful in securing practical ecological observations, and so wishes to give more time to this or to that side of the work, by all means let him do so. Let him teach what he knows best and what he loves best, let him do it entirely in his own way and in his own time. Whatever may be true in other subjects, it is certainly a fact that in biological science the best teaching can never be measured off with a tape-line and delivered by the yard. Nevertheless the time element is most important. It is to be hoped that our biological teachers are none of them animated by the spirit of the narrow pedagogue whose vision is limited by his own subject and who is willing to absorb the whole of a student's time if his fellow teachers will let him. Don't yield to the temptation to make your own subject all there is, but if you are associated with teachers who want the earth, then fight for the thing you teach and fight to win.

It is settled that there are many things in biological teaching not yet settled. Just how we are to develop certain principles and how we are best to secure certain forms of discipline, I have often said if I knew I wouldn't tell. Nothing can ever take the place of good, sound common sense, and no teacher can possibly tell another just how to do it. Scientific teaching is and ought to be experimental. The teacher must have his own high ideals, must study the pupil and the conditions of the school, must use his best judgment day by day, learn by his mistakes, do better the next time, and, like his students, "learn by doing."

University of Michigan. VOLNEY M. SPALDING.

## Helps to High School Teachers of Natural Science.

At a meeting of science teachers held in Rochester, N. Y., in 1900, when asked what preparation in botany they would wish students to have for entering college, the replies were uniform: As things now are, we prefer students to enter college without any study of botany, on account of the hasty and imperfect work. Professors Spalding and Newcombe and others have been talking with little effect on this subject till they are weary. Long since I gave up attempting to reform the world. The trustees or boards of education are the men we should talk to, for they employ the teachers, and they are not here.

I have had a long experience with students who have been taught botany in high schools, and I am sorry to say that I place little value on the work. There are some prominent exceptions. There are great differences in high schools.

In too many cases the teaching of botany is shifted from one person to another without any stability from year to year, with the prevailing notion that any one can teach a class in botany.

In a few cases the class consists of fifty to sixty persons; in most cases the time for the class is a period of only 30 to 45 minutes. Little can be accomplished in this way, even with the best of teachers.

The class should not number over 25 and the period should be at least 90 minutes, consisting mostly of laboratory work. The teacher must have received thorough training in botany, otherwise the topic had better be omitted entirely and something else put in its place. I believe in permitting a teacher to teach what she likes best.

By no means hurry on the start, but go at the study deliberately as though you had all the time you needed. Be just as particular and thorough as you know how to be, of course urging the pupils to study the plants themselves, and not get a lesson in a text-book. The teacher who is compelled to rely on a text-book had better not be entrusted with the work.

If the teacher is a Douglas Campbell he may begin with the study of the lowest forms, using a compound microscope; otherwise, he better not use a compound microscope.

What can the State Academy do to help teachers of science—especially those who have a fundamental knowledge of the topics they attempt to teach?

They may prepare and read papers; they may ask any number of questions; they can listen to the most capable teachers of the state. If any society can help such teachers, it is certain sections of the Academy. This is the place for you; you have found it at last; avail yourself of its privileges; welcome to the section of science teachers! There may and should be at every annual meeting discussions concerning the best new books, apparatus and a teachers' class where methods of demonstration are the prominent feature. W. J. BEAL.

Michigan Agricultural College.

## The Needs of Our Michigan High Schools, as Felt by the Teachers Themselves.

One of a series of questions sent out to the Michigan schools by the chairman of the section on science teaching was as follows: "What do you consider the chief difficulties in the teaching of these sciences?" The sciences referred to were physical geography, physiography, geology, botany, zoölogy and physiology. From replies obtained it would seem that in answering the question the majority of writers had botany and zoölogy chiefly in mind. In some cases principals or superintendents replied, in others high school teachers, thus giving a view of the matter from two standpoints. The difficulties enumerated may in general be included under four heads; insufficient preparation in these subjects on the part of teachers; indifference shown by those in authority; need of illustrative material and laboratory equipment; and lack of time.

An idea of the need for thoroughly trained science teachers may be gained

from statements made by superintendents and principals. The following are some of the opinions given: "The teachers do not know how to go at the work systematically," "They lack a knowledge of scientific facts," "There is a lack of disposition on their part to work up good courses," "They want to use textbooks with minute instructions," "There is a lack of a practical plan of attack on the part of teachers." One superintendent says, "The children are not brought sufficiently to see the living relation between the book and things about them." Another expressed the whole matter well when he said, "The greatest need is live teachers backed up by broad gauge school boards." The trouble at the bottom of all this was well stated by one who said that the preparation of teachers in these lines is "incidental." Even in the instances where the teacher lacks patience, is indifferent, or shows other undesirable traits, we can generally trace the matter to lack of preparation.

In a number of schools the teachers feel hampered because school authorities, parents and pupils fail to see the value of botany, zoölogy, etc. This indifference on the part of those in authority is the chief cause for other difficulties. It explains why schools are not provided with laboratories of some description and laboratory equipment. It also accounts for the fact that no grade work is done in these lines and might, at least in some cases, explain the short laboratory periods. In the northern part of the state the long winters make facilities for indoor culture a desirable thing. Here again wide awake superintendents, principals or school boards would see that such facilities were at hand. Even the matter of time would, I believe, be satisfactorily arranged if the authorities realized the significance of this work. As it is now, in many schools one person is given so many subjects to teach that he either does not give proper attention to any of them, or does thorough work in a few to the neglect of the rest. "One man cannot get material for all the classes," is the statement of one teacher which might be echoed by many others. Under these circumstances teachers must either fall short of their standards of right teaching or overwork themselves. In a few cases it is thought that too many subjects are included in the high school course, and as a result some of the work does not receive proper attention.

The poorly prepared, indifferent teacher is the greatest obstacle in the way of successful work in the natural sciences. Wide awake, enthusiastic, well prepared teachers are the active agents for overcoming all the other difficulties which now present themselves. But while those who have had little or no preparation for the work are forced into it, there is little hope for betterment. In a large number of cases the teacher would do more if he only knew how. For example, there are many teaching the text in Gray's botany with perhaps some analysis and herbarium work, not knowing there is any other way. The pupils come to feel that botany consists largely of hard names. One boy was heard to remark that he did not intend to study it. When asked why, he replied that he had shown his mother all the long, hard names he would have to learn and she had promptly given him permission to omit the subject. The preceptress of this school tells me that the pupils cordially hate botany, while the parents and school board naturally fail to see its value. It is still part of the course simply because it has been for years. The one who teaches it has had practically no



training in the subject and has many other classes for which she is better prepared. Let one who understands plant ecology or physiology go into that school and he would soon place the whole matter on a new basis. Teachers complain that pupils do not observe and it is true that there is a deplorable lack of observational power. But they can be taught to do so, as the natural sciences are especially adapted to train the senses. In fact, it is part of their province to open the eyes. One of the great advantages in this study, especially in our state, is that material is close at hand. The flowers, birds, minerals, etc., are where all can study them. Probably some flowers teachers would like are not easily obtainable, but our common weeds are everywhere and present an interesting field for study. Then we have no lack of trees which can be studied both winter and summer, while many birds and other animals are with us all the time. With only a small expenditure of money, bottles and formalin can be provided in which material collected in its season may be preserved for use in the winter. There is abundant opportunity for ecological and physiological study in both plant and animal world. Such study rightly conducted will not only arouse interest, but will reveal the worth of these sciences. What is needed is fully awakened, well equipped teachers who will take advantage of these things and use them to instill a love of nature in the pupils. When this has been done the interest of parents will naturally follow, for inevitably the children will carry their enthusiasm into the homes. When the pupils and parents have been reached, strong forces have been set in motion for overcoming the indifference of those in authority. Once the value of this science teaching is understood by the authorities, they will be willing to provide necessary equipment and the needed teaching force.

MARY A. GODDARD.

Michigan State Normal School.

## Status of Biology Teaching in Michigan High Schools.

In order to form some basis for just criticism and wholesome advice regarding the matter of the teaching of the Natural Sciences in our public high schools, as well as to establish an exchange bureau for class and museum material, a circular letter was sent to the 224 high school principals of Michigan. One hundred and thirty-five of the blanks have been returned, of which eighty came promptly. The schools replying are, for the most part, the largest and wealthiest of the state, so that the following report, based upon data collected from these blanks, must be taken as a somewhat flattering view of the situation.<sup>1</sup> That improvement has been rapid in the last few years no one doubts, but it is still a very evident fact that the biological sciences are far behind the standard set for the other sciences. It would be out of place to attempt to state here the peculiar value of biology in the lives of our boys and girls, but every teacher, of whatever subject, and every member of a school board ought to make it his or her immediate duty to tabulate the

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<sup>1</sup> The writer begs to state that the percentages given are only approximate owing to the fluctuation and frequent ambiguity of the answers.

educational and ethical values of the study of botany, zoölogy and physiology.<sup>1</sup> Do these subjects demand as much time from teacher and pupil as language work or other sciences? Do they need as carefully prepared teachers? Do they deserve as good equipment?

*First.* What amount of biology teaching (botany, zoölogy and physiology) do our schools provide?

Of one hundred twenty-six schools reporting, seventy-one give one year's instruction. A few of these make it one year of botany, a few divide the year between botany and zoölogy, but the large number, eighty-three per cent., divide the year between botany and physiology. Forty-six of the one hundred twenty-six schools offer one and one-half year's work, of which the far greater number, eighty per cent., divide the work among the three subjects and give one-half year of botany, one-half year of zoölogy, and one-half year of physiology. Only six of the one hundred twenty-six schools give two or two and one-half year's biology teaching. In these schools the subjects are partly elective.

Is it not true that this amount of time is less than for any other group of closely allied subjects? However, if all or most of this time were used in the laboratory there would be less cause for complaint. The fact is, practically all of the physiology work has to be thrown out of consideration because the texts used are so antiquated and because the subject is not presented by laboratory methods. It is and has been a dry, uninteresting, dead, "snap" subject. Note that the university does not now make physiology an entrance requirement. If then we consider only the botany and zoölogy, the amount of time and the teaching force devoted to biology are inadequate.

*Second.* What proportion of the student's time is given to the study of these subjects? How many terms' work has your high school graduate had in biology, the science of life? Compare this amount with the amount of time he has given to the languages, to mathematics, or to the physical sciences. The university requires for entrance that the applicant shall have had one full year's work in either botany or zoölogy, or one-half year of each. This causes the school that wishes its graduates to enter the university without examination to require at least one year's work in botany or zoölogy, so we find in most of the schools that are up to the standard, this amount offered as a minimum. When more than this is offered by a school it is made elective, but it is impossible to always judge from the reports what range of choice is allowed the student. The university fixes the minimum amount of biology required for entrance upon university life; is this amount enough for the boy or girl high school graduate who proposes to enter upon active life? Ought they not to have a deeper knowledge of the inev-

<sup>1</sup> References to the subject of the value of the biological sciences:

1. Home Study Review, September, 1896 (out of print). "The Educational Value of the Biological Sciences." Jacob Reighard.
2. Proceedings of the National Educational Association, 1897. "Zoölogy in the High School Curriculum." H. B. Ward.
3. The Forum, vol. xiv, page 411, December, 1892. "Wherein Popular Education has Failed." President Eliot.
4. Popular Science Monthly, vol. xiv. "Scientific Relation of Sociology to Biology." Jos. LeConte.
5. Social Evolution. Kidd. Chap. 1.
6. New Miscellanies. Chas. Kingsley. "The Study of Natural History."

itable laws that govern health and creation? If they are going back to the farm is there any knowledge that will so enhance their country life as the knowledge of the plants and animals? And ought it not also to be of much practical or utilitarian value? It certainly would be if rightly taught.

Sixty-seven of one hundred twenty-six schools reporting meet the university requirement in biology.<sup>1</sup> There are four ways in which they do this.

(1) Two of the sixty-seven give one year of botany and one-half year of zoölogy. These schools require one semester's work in botany and allow the student to choose either botany or zoölogy for the second semester.

(2) Seven of the sixty-seven offer one year in botany and one year of zoölogy and usually allow the student freedom of choice. The student may then have one full year of either, or one-half year of each.

(3) Sixteen of the sixty-seven offer only one year of botany.

(4) Forty-two of the sixty-seven offer one-half year of botany and one-half year of zoölogy.

Which shall the student choose when it is a matter of election? Which ought the officers to make compulsory, one year of botany, one year of zoölogy, or one-half year of each? A glance shows how the last question has been answered,—the great majority of our schools prefer the combination. Why? In the mind of the writer there are good reasons to be given for both the choice of the full year of continuous work in the one object, either botany or zoölogy, and the choice of one-half year of each. The preparation and the taste of the teacher must help to settle which should be given, for every true teacher must give instruction only in what he can teach well and easily. As for educational value, it is true that the botanical material is easily handled and that it lends itself wonderfully to the needs of careful observational and experimental work and is, therefore, more readily used for purely mind training exercises. But it does not connect itself so closely with the important subject of human physiology, neither does it offer as many or clear illustrations of the theory of development as the zoölogical material. Then, too, the little glance which the student gets into the botanical and zoölogical fields even in but one year's work gives, under good instruction, the wider horizon that is needed to interpret facts and things in every day life; to make intelligent the reading of the papers and magazines and to give the impulse of future work along these lines to the high school graduate who will not have the incentive of college life. The ideal condition would be one full year's work of each, botany and zoölogy. Then if desired the zoölogy could be merged into human physiology in a way that would make that subject full of life, thrilling with interest, and one of the most important in the curriculum. For the sake, then, first, of laying a foundation for human physiology; second, of inculcating the theory of development; third, of giving a broad view of life, I believe both botany and zoölogy should hold equal places in every high school course.

The special plea for zoölogy given above is needed, not because it is more

<sup>1</sup> The university requirements in these sciences for entrance are: Botany: Laboratory work for one school year; or, Zoölogy: Laboratory work for one school year; or, Biology: one-half year of botany and one-half year of zoölogy.

important than its sister, botany, but because its place is not fully recognized. This may be due to the fact that botany is already in the schools and there seems no room for zoölogy, but it is probably because of the greater ease with which botanical material is manipulated. Certain it is, botany is much the more popular of the two. Out of the first one hundred and five schools that reported, about half give botany and no zoölogy and the other half give botany and zoölogy, making ninety-nine per cent. giving botany, while only fifty per cent. give zoölogy. There are no schools that give zoölogy alone, and of those that do offer it the great majority have but one-half year's (twenty weeks') work. Only three schools, of all reporting, give no botany or zoölogy whatever. These are Iron Mountain, Ironwood, and Calumet. In Iron Mountain a strong physiology course, running through the second year, is given, but no actual laboratory work is done by the pupils and with only one compound microscope not much insight can be had of the fine structure of working tissues.

The ideal condition was spoken of above. Of all the schools heard from perhaps the one that most nearly approaches this ideal condition, in the writer's mind, is the Jackson West Side High School. Botany is given in the entire first year,—followed in the second year by zoölogy-physiology, which is also a forty weeks' course. In reporting on this the instructor said: "Zoölogy, so called, is pursued for a year, and man is the last of the types studied. After thirty or thirty-five weeks' work on the lower forms of animal life, a great deal can be done in human anatomy, physiology and hygiene in a much shorter time than when the subject of physiology, so called, is taken separately." Bergin's *Foundations of Botany* is used in the botany course and both Kingsley's *Comparative Zoölogy* and Martin's *Human Body*, Briefer Course, are used in the second year's work. These courses are elective so that the student may have one of three options: (a) one year of botany; (b) one year of zoölogy-physiology; (c) one-half year of each.

*Third.* As to the years in which the biological sciences occur in the curriculum. Nearly all the botany classes are about equally divided between the first and second years. Zoölogy is also placed early in the course, usually in the second year. There is a tendency to let zoölogy follow botany, a very natural sequence. Physiology is often placed in the first year. Many schools report it only in the eighth grade. If physiology is to be taught by itself and as pure physiology, it deserves to be, and must be if thoroughly mastered, placed after botany, zoölogy, and chemistry. Probably the courses given thus early should simply be called hygiene. The place assigned to botany and zoölogy is not adversely criticised but commended, because the character of the material naturally makes it precede the more complex subjects of history, political science, literature and art. To quote from Professor Reighard's paper: "The place I would assign to the elements of biology taught in a secondary school, is as a training for all those studies that have to do with living things; introductory then to history, physiology, and social science, and of incalculable benefit to every man whatever is to be his future occupation."

*Fourth.* Methods. The laboratory method of course prevails in botany. Only sixteen per cent. of the schools reporting give no laboratory work in botany.



So also in zoölogy, although many classes are making the zoölogy merely the reading of Jordan & Kellogg's Animal Life. In physiology only a few report definite laboratory work. The physiology courses of the Jackson, Iron Mountain, and Detroit Eastern High School are to be mentioned among some of the best. Of the botany classes, about forty-three per cent. devote more than half their time, to laboratory work, but about fifty-seven per cent. give only one-half their time, or less, to laboratory practice. Three-fifths the time is usually considered necessary for careful work. As for field work, a special conference should be held on that subject alone. It is certainly an encouraging sign that an effort is made on the part of many teachers to take the pupils to the field and woods for actual observational work. Twenty-five per cent. of the botany classes report some such regular work. How to conduct the parties successfully, how to get the results, is the problem yet to be settled for most of us. That it can be done has been attested by a few.

*Fifth.* Length of class and laboratory periods. Of the botany classes reporting on this question eighteen per cent. have periods of thirty-five minutes or less; seventy-one per cent. have periods ranging from forty to fifty minutes; and seven per cent. have as long as fifty-five minutes or more. Good results will never be obtained from laboratory work until at least sixty consecutive minutes can be employed by the pupils. The getting out and putting away of material alone consumes much time, and when perhaps only thirty minutes are left for observation, notes, drawings and personal quizzing, the results are scattered and erroneous. The best work comes with a feeling of plenty of time to do most careful and thorough observation and a chance to "talk it over" with the instructor. Laboratory teaching is at best a slow method of imparting knowledge, but it is supposed to be the natural and sure one. Let us give it a fair chance by allowing both pupils and teachers more time.

*Sixth.* Texts. Bergen is by far the favorite botany text. Coulton is used in many schools and a few, say six per cent., still use Gray alone. In zoölogy, Jordan & Kellogg's Animal Life receives the largest number of votes and Needham stands a close second. They are often used together. Burnett seems to be the next most popular manual. A few schools still use Steel!

*Seventh.* Equipment. It is hard to judge of the facilities for laboratory work from the reports. Perhaps the best index is the number of compound microscopes, since it usually indicates the amount and degree of laboratory work attempted. At least ten per cent. of the first one hundred schools replying have no compound microscope at all, about one-half have but one, and only fifteen per cent. have more than five. The best equipped high schools seem to be able to do a good grade of laboratory work with, say, fifteen microscopes by dividing the class into small sections, which should not at any time consist of more than fifteen or twenty pupils.

*Eighth.* General preparation of the instructors. The general, not necessarily the special, preparation which the instructor received is shown by the college which he attended. Of the first one hundred reporting, twelve per cent. came from institutions outside the state; twenty-four per cent. received their education at the university, thirty-six per cent. are normal school students; and

twenty-three attended other colleges in the state. A few say they are self taught.

*Ninth.* Preparation of the student. Of elementary science in the grades, fifty per cent. of the schools do "a little," twenty-one per cent. do regular nature study work; and the remaining twenty-nine per cent. do none. One school, Menominee, has a high school course called nature study. The principal, and the instructor, Mr. W. L. German, writes that this course has proved so much of a success that it is to be continued. He says, quoting from a letter of his: "The grade work has not been sufficient to enable our high school to begin science work upon a fair basis. It occurred to us that a year's work could be planned that would round out what had already been done, that would lead the pupil into a correct method in science work, and that would lead up to the after work of our courses. My talks and observation lessons have been along three lines—physiography, botany, and physics. My present class is composed largely of boys. I have studied them, that I might find their lines of interest, their needs. I have studied nature, the world just about us in all of its aspects, that I might bring it indoors or take them outside to see, to feel, and to know it. At the middle of the year I took in several first year boys who were seeming failures elsewhere. They have in every case made a success of nature study. These boys tell me, "We like this hour better than any other in school." The subject is one that will enable us here to hold boys right on to the end of the year." This first year of preparatory work is followed in the second year by physical geography and physiology. Botany and physics, each full year subjects, come in the third year, and the year of chemistry is placed in the fourth year of the high school course.

Will the time soon come when our boys and girls, after being in the school eight years, will know all the forest trees, all the wild flowers and weeds, all the flowers and vegetables of the garden and their general mode of life, and the relation of flower to fruit? Will they also know the haunts and habits of our native animals and be able to recognize our list of about seventy common birds and tell what they are good for? Will they be able to tell the direction of the wind and its possible shifting? Will they know a toad from a frog and the poison ivy from the woodbine? When they do we shall be able to extend our biology courses in the high school, and our young people will have sharper eyes, keener ears, stronger lungs, and a deeper enjoyment in the big out-of-doors which belongs to them. Perchance too they will have a more rational view of life.

#### SUMMARY.

Most of our schools give a fair amount of time to the teaching of the three biological subjects if it were all utilized. The physiology, owing perhaps to the nature of the material, is rarely ever given in a manner that allows that subject to be counted. Botany and zoölogy are the biological sciences recognized by the university in its entrance requirements. A fair consideration of the condition of these two subjects in our high schools brings us to the conclusion that too little time and too little equipment is devoted to them to allow of solid labo-

ratory work or to prepare the pupil for future appreciation and use of the natural world.

Of the two sciences botany is the more popular, but does not in the opinion of the writer offer alone the material needed for all-round training. The majority of the schools prefer a combination of the two. The ideal condition is one full year's work of botany and one full year's work of zoölogy preceded by a general and popular course of elementary science in the grades. The physiology work might be combined with the zoölogy.

Most of the biology work is given in the first half of the course, where it belongs. Laboratory work prevails and there is a growing demand for field work.

The class and laboratory periods are too short to allow of efficient work.

The elementary science of the grades does not give the preparation for high school science or for general life that it ought.

The thing most needed and really demanded, I think, is the popularizing of science. Books and papers are doing much, but illustrated lectures, exhibits, and entertainments might be given by the schools that would arouse the attention of our patrons. Perhaps the exchange bureau suggested by Professor Sherzer will help.

JESSIE PHELPS.

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## The Greatest Present Need in High School Work.

Aside from the importance of properly trained teachers, endowed with enthusiasm and common sense, the greatest need in biological teaching in the high schools to-day is less anatomy and morphology and more natural history and ecology. The prime object of a course in either zoölogy or botany in the high school, aside from its general educational value, ought to be to make the pupils love animals and plants, and find in them their friends. Every student ought to feel when he sees a new flower or animal that he has made a new friend, and each spring and summer ought to bring the renewal of countless old friendships. The high school work in biology ought not to attempt to replace a college course, but should be introductory to the whole subject, leaving the details of morphology to the later course.

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A USEFUL LIGHT FOR BIOLOGICAL LABORATORIES.—Acetylene lamps have some advantages over other artificial lights for use with the microscope when good daylight is not available. These are: less irritating character of the light, greater whiteness, that enables color to be justly judged, and portability. With these is joined an intensity sufficient for use with Zeiss 18-ocular and 2-mm. objective.

The best lamp for individual and for class use seems to be the acetylene lamp known as the "Electrolite." To adapt this to microscopic work we add a "bobeche" as used for Welsbach lights, made of finely ground imported glass. There is also added an opaque shade, instead of a globe, large enough to restrict the light to the area of the table in use. All the light used passes through the ground glass and is diffused.

For use with high powers and vertical stand the too tall lamp may be placed lower than the work table. On the other hand, for a class using low powers the tall stand will spread the light, so that ten or twelve may work around one lamp if the tables are properly placed.

The objection to acetylene lamps is the trouble of attending to them, but in the "Electrolite" filling and cleaning are not difficult, and with one change of carbide the lamp may be put out and relighted at any time till more than ten hours of actual burning have passed. Acetylene for microscopic work has been commended by the Canadian pathologist, Chas. H. Higgins.—*Science*.

## Planarians.

Planarians belong to the phylum *Platyhelminthes*, which includes the most simply organized worms. The *Platyhelminthes* are divided into three main branches. These are: (1) *Turbellaria*, (2) *Trematoda*, (3) *Cestoda*. The planarians fall within the first of these main branches, viz., the *Turbellaria*. The *Turbellaria* are usually divided by systematists into three principal groups: the *Polycladida*, the *Tricladida* and the *Rhabdocœlida*. It is with representatives of the last two groups, the triclads and rhabdocœles, that we are concerned here. Numerous representatives of both these groups are found very commonly in fresh water over a large portion of the globe. The fauna of Michigan is especially rich in representatives of both these groups.

It is the purpose of these notes to point out to teachers the importance of these organisms in teaching work, and to suggest some specific uses to which they may be put. Hints regarding their collection will also be given.

### (1) *Planarians found in Michigan.*

We have in Michigan a number of species of the family *Planariidæ*, or planarians *sensu strictu*. The most common representative of the family in the southern part of the state is the well known *Planaria maculata*. This is a small worm, mottled grayish-brown in color, ranging in length from 5 mm. to 20 mm. (approximately). It is roughly cigar shaped in outline and is markedly flattened in the dorso-ventral direction. Besides *P. maculata* several other species of the genus *Planaria* are commonly found in the state. The genus *Dendrocœlum* is represented by at least one species.

The forms just mentioned all belong to the group *Tricladida*. No systematic study of the rhabdocœle fauna of the state has ever been attempted, so that only an approximate statement regarding their occurrence can be made. A considerable number of species, representing several of the families of the *Rhabdocœlida*, have been found by the writer in the region about Ann Arbor, and there is no reason to suppose that these forms are more abundant in this than in other parts of the state.

In general it may be said that there is little doubt that any teacher in the state can, with very little trouble, collect in his immediate locality representatives of a number of species, both of the *Tricladida* and the *Rhabdocœlida*. It should be added that wherever planarians are found at all they are almost invariably found in large numbers. Hundreds of specimens of *Planaria* may be collected in a few hours after one has found a locality where they occur.

### (2) *The Collection and Care of Planarians.*

The common species of triclads (*Planaria maculata* and other species of *Planaria*) are most usually found on the under side of stones and bottom debris, in not too rapidly running water. Portions of rivers and smaller streams where the bottom is covered with stones from two or three inches up to a foot in diameter will usually yield planarians in large numbers. *Dendrocœlum* is more usually found in pools of stagnant water, as are almost most of the rhabdocœles.



In collecting specimens of *Planaria* one can get the best results by wading into the stream and picking up the stones from the bottom and examining their under surfaces. Where the collecting is good twenty or more specimens of *Planaria* may be found on the under surface of a single stone not over three inches in diameter. The specimens may be most expeditiously removed from the stones by gently slipping a thin spatula or section lifter under their bodies and lifting them off in this way. The specimens may then be shaken off the section lifter into a bottle of clean water for transportation to the laboratory.

Rhabdocœles may be obtained in numbers by taking a quantity of plant material from a stagnant pool and then putting this into culture dishes with clean water in the laboratory. The rhabdocœles will soon appear at the surface of the culture and may be taken off with a pipette. Rhabdocœles may also be collected with a Birge net in places where they are abundant,

All planarians live very well in aquaria in the laboratory. The only precaution necessary is to see that there is not too much decaying vegetable or animal material in the aquarium dish. Planarians will eat voraciously the crushed bodies of fresh water mollusks, as for example, *Physa* or *Limnea*. The ease with which planarians may be kept alive in the laboratory makes them very useful objects for the teacher.

The best killing and fixing fluid for planarians, in case one wishes to preserve them, is *corrosive acetic*.

Formula :

Saturated aqueous solution of corrosive sublimate	95 parts.
Glacial acetic acid        -        -        -        -        -        -	5 "

Fix for one-half hour; wash in 70 per cent. alcohol-tincture of iodine. Avoid touching the fluid or objects with metal.

After fixation in this fluid the specimens should be preserved in 70 per cent. alcohol.

It is apparently impossible to kill planarians in a fully extended condition, and in general preserved specimens are much less satisfactory than living. Since planarians may be collected and kept alive in the laboratory so easily there is very little reason for preserving them unless one wishes to study their histological structure.

They may be shipped alive for purposes of identification if they are placed in a vial of perfectly *clean*, filtered tap-water, without any other material, either animal or vegetable. No air space should be left at the top of the vial between water and cork. Such vials may be shipped by mail in any of the regular mailing cases adapted to the transportation of fluids.

(3) *Uses to which Planarians may be put by the Teacher.*

A. To demonstrate the process of regeneration.

Planarians have a very marked capacity for reproducing or regenerating parts of the body lost through accident, or as the result of operative procedure. If the head of a planarian be cut off a new head will be formed in a comparatively short time. This is an extremely significant physiological phenomenon and one which cannot fail to arouse the interest of students. The experiments

are very easily performed, and if plenty of clear water surrounds the cut pieces of *Planaria* they will almost invariably be successful.

B. As a morphological type.

Planarians are comparatively simply organized Metazoa, and are representatives of the simplest worms. They are well adapted for study as a morphological type form, wherever a compound microscope is available. They have represented in the simplest condition all the organ systems—nervous, digestive, reproductive, excretory, etc.,—found in the higher worms, such as for example the earthworm.

C. To demonstrate reproduction by fission in a metazoan.

Many planarians regularly reproduce asexually by transverse fission. If specimens are isolated under favorable conditions they will be found to divide transversely. In these products of fission the missing parts are subsequently regenerated.

Other uses, besides these suggested here, to which planarians may be put in teaching work will readily occur to teachers who have them in the laboratory.

(4) *Readily Accessible Literature dealing with Planarians.*

Excellent accounts of the anatomy, habits, methods of reproduction, distribution and taxonomy of planarians are to be found in :

1. The Cambridge Natural History. Volume II. New York (The Macmillan Company). 1896. Price, \$3.50 net.

The part of this volume dealing with planarians is contributed by Gamble, and is probably the best general account of these forms in existence.

2. Parker, T. J., and Haswell, W. A. A Text-book of Zoölogy. 2 Vols. New York (The Macmillan Company). 1899. Price, \$9.00.

A very good account of the biology of planarians is included in the first volume of this work.

The following works deal with special topics regarding planarians :

1. Morgan, T. H. Regeneration. New York (The Macmillan Company). 1901. (Columbia University Biological Series, vii.) Price, \$3.00.

In this work is summarized practically all that is known regarding regeneration in planarians. Anyone interested in this subject should consult this work.

2. Pearl, R. The Movements and Reactions of Fresh Water Planarians; A Study in Animal Behavior. Quarterly Journal of Microscopical Science. N. S., Vol. 46, pp. 509-714, 1903.

Discusses the habits and behavior of planarians.

3. Curtis, W. C. The Life History, the Normal Fission and the Reproductive Organs of *Planaria maculata*. Proceedings of the Boston Society of Natural History. Vol. 30, No. 7, pp. 515-550, pl. 9-19, 1902.

Discusses the methods of reproduction in *Planaria*.

University of Michigan.

RAYMOND PEARL.

## Instructions for Collecting Insects.

First catch your specimen, if a bug or beetle, simply put it into a cyanide-bottle or in a bottle having some strips of blotting-paper moistened with chloroform. If a butterfly or large moth, catch it in a net and gently pinch the thorax between the thumb and finger, one on each side of the thorax, until the fluttering either ceases or becomes feeble, and drop into the killing-bottle and leave for several hours. If a bee or wasp, then more care may be necessary. Flies should be kept in a separate bottle and only a few should be put together, as they are delicate and become mussy very readily. Butterflies and moths lose some of the scales from the wings and bodies, and these scales are apt to become attached to other insects, so keep all such specimens by themselves.

A cyanide-bottle is a constant source of danger and should never be made or used except by responsible persons. It is made as follows: A large mouth bottle is selected, usually a six-ounce bottle such as is used for quinine. On the bottom drop one or two pieces of cyanide of potassium, about half as large as a hickory nut in all. Cover these pieces of cyanide with dry plaster and over all pour a layer of plaster freshly mixed with water to the consistency of thick cream. This upper shell of hard plaster should be a good quarter of an inch thick and should be free from bubbles. Gently tap the bottle on the ground while hardening to get rid of the bubbles. Place the newly filled bottle in a cool, dry place out of the sun, for a few hours, and then, when it is dry, place the stopper in position and keep it stoppered on all occasions except when putting in or taking out insects. Never put a bottle in the sun, for fear that it will become moist, and do not put insect-pins in the bottle, for the fumes of cyanide attack the german-silver of the pins and later they are sure to corrode.

### MOUNTING OR PINNING.

Bugs, grasshoppers, large Hymenoptera, and flies should be pinned through the thorax, passing the pin perpendicular to the long axis of the body and allowing just one-fourth of its length, from the head to the point, to project above the insect. Beneath the insect, and spaced so that it easily may be read, should be placed the locality and date label. Always put this label on when the insect is pinned. The locality and date label should be printed or written with drawing-ink (which will not fade) and it should be very small. Small parasitic Hymenoptera are fastened by thick shellac to paper points and these points are pinned. Beetles should be pinned through the right elytron or wing-cover near the median line and at a point about one-fourth the distance from the base to the tip. Moths and butterflies should be stretched or spread on specially made spreading-boards. This is to render the wings flat and to expose the desired parts to view. The figure shows the manner of spreading. The board has a channel running lengthwise and on the underside of the channel is fastened a strip of corn-pith in which the pin may be set. The wings are carefully pulled forward with a pin and a thin sheet of mica is placed over them and pinned down with mourning-pins. In doing this, the hind margins of the front wings should form a straight line. The hind wings should

be brought up so that there is no large space between them and the front wings, merely a V-shaped notch at the sides.

A net is a very desirable accessory for a collector. The ring should be made of stout galvanized steel wire, fastened preferably with solder in a socket and fitted with a light handle three or four feet long. The ring should be about ten inches in diameter and the bag of cheese-cloth just a little shorter than the arm, so that one can easily reach to the bottom. The bottom should be rounded so that no vexatious corners offer hiding places for the specimens.

The standard pins are known as Klaeger pins. They are made of german-silver and vary in size. Number 3 is the most useful although No. 5 is very convenient for large insects and No. 1 for small forms.

By far the most interesting and instructive collection is made by rearing the insects from the egg or larval condition. Caterpillars, grubs, and larvae of all sorts usually may be reared or bred by placing them on their appropriate food-plant in cages. A small cage can be made by embedding a bottle for water in the soil of a flower pot. Twigs of the food-plants may be placed in the bottle and replaced, when wilted, by fresh twigs. Over the food should be placed an open glass cylinder to enclose the whole, or if these are not to be had, use a lantern-globe with a piece of Swiss muslin tied over the top of the globe or cylinder for ventilation. The muslin may be tied on with a string. If a colony of caterpillars be started, specimens should be saved, from time to time, at the various stages and preserved in wet form in bottles. A very satisfactory preservative is made by mixing formalin, alcohol, and water as follows:

Alcohol (95 per cent.),	-	-	-	-	100 c. c.
Water (rain or distilled),	-	-	-	-	100 c. c.
Formalin (40 per cent formaldehyde),	-	-	-	-	10 c. c.

Such reared specimens possess an interest far beyond that of specimens merely caught at random, and this is especially true if the specimens are accompanied by careful notes.

Insects may be sent in cigar boxes lined with cork. If a layer of split cotton wadding be pasted over the cork, it will catch any legs, antennæ, or wings that may become jarred off in transit. The cigar boxes should be wrapped in cotton-batting and then in paper, or else a small box should be enclosed in a larger one with cotton firmly, but not too tightly, packed between.

All this and very much more can be found in Professor Comstock's "Insect Life," published by Appleton of New York. No better beginning could be made by one intending to commence the study of insects, than by obtaining a copy of this book. Directions for inflating larvæ and preserving all sorts of insects, with advice as to cases, etc., are fully given with many illustrations.

Supplies may be obtained of many dealers. The American Entomological Co., 1040 DeKalb avenue, of Brooklyn, and Bausch & Lomb Optical Co. of Rochester, N. Y., and many others, supply the goods at standard prices.

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RUFUS H. PETTIT.



## Directions in Regard to Shipping Reptiles and Amphibians.

Wherever possible, these animals should be sent alive. If small, they may be placed in a box with moist paper, leaves, moss, cotton-batten, or any similar substance that will keep them from shaking around; send by mail. They require little air, and unless the package is hermetically sealed (which it should not be) they will live thus for some time. Larger specimens must be sent by express, and such cases should always be preceded by a letter; even a rough description will oftentimes make it unnecessary to send the specimen. If the animals are dead, the only thing to do is to make a slit in the ventral side, deep enough to open the body-cavity, and put them in alcohol or formalin (4 per cent. solution). In exceptional cases, when neither alcohol nor formalin is available, they may be injected with a solution of carbolic acid, put into a small box as air tight as possible, and sent by mail *at once*. Specimens in liquids can be sent by mail if in approved packages, but will usually have to be sent by express. In all cases name and address of sender should be on the outside of the package.

Olivet College.

HUBERT LYMAN CLARK.

## Hints on Collecting Land and Fresh-Water Mollusca.<sup>1</sup>

### COLLECTING APPARATUS.

For land shells, a "Ferriss" hoe is very useful. This is made by getting a small, light-handled garden hoe and having the blade cut down at a machine shop. It should be about three inches wide on top and taper to a sharp point. Then cut off the handle so that it will be as long as a cane. This makes a most convenient tool for turning over logs and breaking up rotten wood and digging around stumps and among the dead leaves. A pair of fine curved-pointed collecting forceps is also necessary for picking up the small species. Small glass bottles should be carried, as the small species are apt to get lost in the dirt and slime, if put into the same receptacle as the larger ones. It is better not to put the small species in alcohol as they are collected, as they are then killed at once with the animal more or less extended. If put in a dry bottle and left a few hours they will withdraw into their shells, leaving the aperture clear and fit for examination. This is especially necessary with the *Pupidae*, where the arrangement of the apertural teeth is a specific characteristic.

For the larger species tin cases of a convenient size to slip readily into the coat pockets are most convenient. Several boxes and a number of vials should always be carried, so that specimens from different localities may be kept separate.

For the fluviatile species it is necessary to have a dipper. This is made from an ordinary tin one, by removing the bottom and substituting one of fine wire cloth. By removing the end of the handle, the dipper can be slipped on

<sup>1</sup> Abstract from article which appeared in JOUR. APP. MICRO. AND LAB. METH., page 1954.

to the end of a cane or pole when in use. This is useful not only for reaching the larger specimens from the shore or boat, but especially for sifting the mud and sand from the bottom, where a multitude of small species live, which otherwise would not be found. It will be found more convenient to empty the contents of the dipper, when thoroughly washed out, into a pail and carry the whole mass home before undertaking to pick out the shells. If attempted in the field, many of the smaller and more desirable things are apt to be overlooked. By spreading the mass out in the sun for a short time it will become dry and friable so that the shells can be easily separated and picked out. An ordinary reading glass is very useful for the detection of the more minute forms in sorting over such material.

#### WHERE TO COLLECT.

Everywhere. The land species love dampness and darkness. They are to be looked for under logs, bark, and leaves in suitable localities. Many species bury themselves in rotten logs, and these should be broken up with the hoe. The accumulation of dead leaves around fallen trees is a favorite habitat and should also be carefully and slowly gone over with fingers and hoe. The thick grass and dense thickets along the margin of ditches and streams will usually reward a careful examination. Southern and western exposures, being dryer, are not so fruitful as eastern and northern hillsides and shady ravines. Coniferous forests are usually quite barren of molluscan life. An open hardwood forest in a limestone region is the ideal hunting ground. Nearly every permanent body of water has its mollusks, varying according to its character. Some species are found only in rapid flowing water, and others only in ponds and still water. Ditches and other stagnant waters are usually good collecting ground for *Pisidia* and other small species. The low places in the woods, which dry up in the summer time, have a number of species that are not found elsewhere, and which bury themselves in the mud when the water disappears. Sand banks in rivers and lakes are the favorite resort of many of the smaller species. The under side of the lily pads should be scrutinized, while the *Ancyl*i should be looked for on stones and dead clam shells.

#### CLEANING AND PREPARATION OF SPECIMENS.

The larger *Helices* should not be put into alcohol unless desired for anatomical purposes, as it is almost impossible to remove the animal after it has become hardened. They should be boiled as soon as possible. The water should be boiling, not simply hot. Species of about the same size should be boiled together in order that the operation may be successful. A small wire strainer with a long handle is very convenient for holding the snails while boiling. If dropped directly into the water, there is apt to be trouble in fishing them out and they are likely to be boiled too much. The time varies according to the size and the species, some requiring more time than others. If not boiled enough, the muscular attachment to the shell will not be loosened, and the animal will not "pull" at all. If boiled too long, it is apt to break in two and give a good deal of trouble before extraction. The time required varies from ten seconds for a species of the size of *Polygyra monodon* to sixty seconds for

*P. albolabis*. It is well to experiment a little at first with a specimen or two of each kind until the proper time is found. Only a few should be boiled at a time, as they "pull" easier while warm. When boiled, the animal should be slowly and carefully pulled out. Too much haste is apt to cause the animal to break apart, leaving the apical whorls still in the shell. The curved points of the collecting forceps are convenient for extracting the animals and hooks of various sizes can be made from safety pins. By tying these on to small wooden handles very effective instruments can be made. A small, fine-pointed dental syringe is indispensable for this work. If the animal cannot be started with the hook, or if it breaks in two, a jet of water from the syringe will usually solve the difficulty. In case very desirable specimens get into this predicament, putting them in alcohol for twenty-four hours will contract the remnant of the animal sufficiently to enable the successful use of the syringe. Many of the species have the aperture so obstructed with teeth, that it is difficult to extract the animal with the hook. In such case a vigorous use of the syringe will force enough of the body out of the shell to enable the hook to be used. When the animal is completely extracted, the interior should be thoroughly washed out with the syringe. A small piece of sponge on the end of a fine copper wire, which can be bent in any direction, is very useful for removing the mucous, which is apt to adhere to the interior of the shell. This should always be carefully attended to, as it will greatly disfigure the specimen when dried. The exterior should then be thoroughly scrubbed with a soft tooth or nail-brush. No oil or acid should be used on any of the land shells. It is not desirable to attempt to clean the small species by removing the animals. By keeping them for a short time in a dry place, the animal will retire far within the shell. Then they should be put into 25 per cent. alcohol for a day or two. If to be left longer in the alcohol, the strength should be increased. Twenty-four hours, however, in the alcohol is all that is necessary. Then they can be dried in the air without leaving any offensive odor. Either before or after drying they can be cleaned by putting them in a bottle with some fine, clean sand and shaking them together until all the dirt has been removed by the sand.

With the exception of the larger species of *Planorbis*, which are more easily cleaned by boiling, it is practically immaterial whether the fluviatile univalves are boiled or put directly into diluted alcohol. In either case there is no difficulty in extracting the animals. The minute species are treated the same as the small land shells. In the operculate species, it is desirable to retain the opercula of, at least, part of the specimens. These are easily removed from the animal and, after being cleaned, should be put inside the shell and the aperture plugged with cotton. All the foreign matter both inside and outside of the shell should be removed by thorough washing. All the water species are apt to be more or less incrustated with deposits of lime or oxide of iron. These can be removed by immersing them in oxalic acid. Care should be taken not to prolong the operation, or the texture of the shell may be injured. The *Ancylis* are always more or less coated in this way, and can easily be cleaned by floating them for a few seconds on the acid, upside down, and then gently brushing them off with a soft brush while held on the tip of the finger.

The larger bivalves should be well washed and, if necessary, scraped off with the knife as soon as taken, care being taken not to injure the epidermis.

They can be boiled, if desired, when the shells will open and the animals easily removed. But as a rule it is more convenient to cut the muscles, which hold the valves together, with a thin bladed knife and scrape the animal out. Care should be taken not to break the edge of the fragile species when inserting the knife. All traces of animal matter should be removed, and after a thorough washing the valves tied together with a string until thoroughly dried. Never use colored twine for this purpose, as it is apt to stain the shells. Any surface incrustation can be removed either with oxalic or muriatic acid. The latter is more convenient for the larger species, and can be applied with a small brush. It does not bite the fingers, so that it can be used freely. Care, however, must be exercised in using it and the specimens frequently washed, lest damage be done to the shell. The smaller bivalves, the *Sphaeria* and *Pisidia*, are best treated by putting into diluted alcohol for a day or two and then drying them. If left too long the shells are apt to open, which interferes with the looks of the specimens.

The larger species of *Sphaerium* are better with the animal removed. This can be done after boiling or a few day in alcohol. As these are usually too small to be easily tied together to keep the valves from gaping, each specimen, while the hinge is flexible, should be closely wrapped up in a small piece of tissue paper until completely dry.

Both in collecting and cleaning, the specimens from each locality should be kept carefully separated and labeled. Too much importance cannot be given to this point. The study of the geographical distribution of the mollusca is one of the most important branches of conchological work, and this, to be of any value, must be based on absolutely accurate work on the part of the collector.

#### PACKING SPECIMENS.

Small specimens should not be mixed with large ones, as they are apt to get lost; nor should fragile shells be put in with stronger ones, as they are likely to be broken. The minute specimens can be put into gelatine capsules, small vials, quills or paper tubes made by rolling writing paper around a lead pencil, gumming down the edge and stopping the ends with cotton. Don't mix shells from different localities. Write the locality on a label and wrap it up with each vial or package. Use plenty of cotton in packing fragile shells. Pill boxes and match boxes are convenient for packing purposes. Wrap up *each* vial or box separately, then if a smash does occur there is a fair chance of saving some of the specimens and no danger of mixing the contents of different packages. Don't send paper boxes by mail. It is simply tempting Providence. Pack in a wooden box.

BRYANT WALKER.

Detroit, Mich.



## Some Suggestions for the Beginner in Collecting and Studying Fleshy Fungi.

Few persons in our state have any idea of the variety and extent of our fungus flora. And while this is partly due to the inconspicuous nature of these organisms, it is largely dependent on the fact that they are usually passed by without attention or shunned as being loathsome or dangerous. But the person who once becomes interested in studying these lowly plants, will soon be led to admire their dainty forms and delicate colorings, and to marvel at their wondrous adaptations to some special mode of existence. Moreover, many of the native fungi of this state are capable of furnishing a highly prized article of food, which fact is sufficiently interesting to many persons to make this subject an alluring one.

There is an awakening interest in the study of these lower forms of plant life, however, and an increasing desire for information concerning them. That this knowledge should be disseminated by means of the teachers and through the common schools of our state seems a natural process. Hence, it is with the intention of giving the beginner a general idea of the nature of fungi, as well as some hints as to how to go to work along this line, that these suggestions are presented.

### THE NATURE OF FUNGI.

Briefly stated, fungi are plants of a low order. They are quite closely related to the sea weeds and the green pond-scums of fresh water, all of which are known as algæ. The fungi, however, differ from these plants, and from most of the higher or flowering plants, in the total absence of the green coloring matter (chlorophyll), found in the leaves and young stems of such plants. This green coloring matter enables the plants possessing it to assimilate the raw food materials of the soil and air and thus to build up their tissues on inorganic matter. The fungi, consequently, are unable to live and grow in this manner, but must obtain their nourishment from substances which have been previously organized, or from organic matter. A great many fungi exist wholly at the expense of living plants, animals, or insects, thus forming true parasites. Thus, many of the most destructive diseases of our cultivated plants are due to the attacks of these parasitic species of fungi, such as the mildews, rusts, smuts, and blights.

A large number of fungi, however, live on decaying organic matter, such as rotting wood and leaves, straw, manure, and the humus in the soil, thus acting the part of scavengers. It is among the members of this class that we find the largest and most showy kinds of fungi, many of which are edible while a number are known to be very poisonous when eaten.

In its early stages of growth a fungus consists of a very delicate, branching system of cobweb-like filaments or threads, which spread in all directions, in search of nourishment, in very much the same manner as the fine rootlets of other plants. This network of slender threads is known as mycelium or spawn, and serves the purpose of roots and stem. It may be observed by overturning pieces of decaying wood or rotting leaves in the woods, or it can be cultivated

on a piece of bread or other cooked food by keeping it for several days in a closed dish and in a warm, moist air. Several common moulds are sure to appear under such conditions, and will serve to illustrate the appearance and growth of this mycelium. In the case of the larger fungi the mycelium often forms quite large strands or felt-like sheets of interwoven threads, and later these may give rise to the fruiting part of the fungus in the form of a mushroom.

Fungi are reproduced by means of microscopic cells called spores, which are produced in immense numbers on the mature or fruiting part of the fungus. These spores are so light and small that they float readily in the air and are spread or disseminated in this manner. By squeezing a dry puff-ball a little cloud of smoke-like spore dust is given off and if a little of this dust be examined with a powerful microscope the spores may be observed.

When the spores of fungi are surrounded by favorable conditions of moisture and heat they will germinate by pushing out a minute tube, which by continued growth and branching forms the mycelium.

#### COLLECTING FUNGI.

In collecting those fungi which live as parasites on other plants, it is customary to take a portion of the affected plant, called the host plant, and dry it carefully, usually in a plant press, making notes as to date, locality, host plant, and collector. Thus pieces of diseased stems, leaves, and fruits may be collected and later examined for the name of the fungus. This work, however, requires the use of a compound microscope and some training in its use, consequently these suggestions will apply mostly to the collecting and studying of the larger fleshy fungi.

The most favorable time for collecting these fungi is during the warm, rainy weather of spring, summer, and autumn. A few kinds may be found even in winter when the temperature rises above the freezing point, but these are confined principally to decaying logs and stumps, or the trunks of trees. Some kinds occur in dooryards and pastures or along the road side, while a few thrive in gardens and cultivated fields, but the majority are to be sought for in rich, moist woods and in grassy openings in such woods.

Some arise from soil where the mycelium has been growing unseen for a long time; others spring up at the base of stumps and trunks, while many thrust themselves out on the surface of some old log, fallen branch, or dead tree, and many kinds find a natural mushroom bed in the decaying leaves of the forest. For collecting fleshy fungi only a few simple tools are needed. A market basket with several small tin or pasteboard boxes, for the smaller specimens, and an old butcher knife or small trowel for lifting them from the soil or prying them from logs, will suffice; care should be taken to secure all parts of those kinds which grow on the ground. Thus they should be lifted by digging under the base of the stem, and not pulled up. Specimens intended for study should be carefully handled so as not to break or rub them. The woody kinds which grow on decaying timber can usually be dried at once and studied later, although it is desirable to secure some of the spores in the form of a spore-print, described later. The more perishable kinds should be studied in as fresh condition as

possible, as many important characters, by which they may be identified, are lost on drying.

The following notes will assist in recognizing the commoner groups of fleshy fungi:

*The Morels*—These fungi appear in spring. They consist of a hollow stem with a sponge-like cap or head, the surface of which is covered with wrinkles and pits. The spores are produced in slender cells which cover the folds or wrinkles on the caps.

*Cup-Fungi*—There are a large number of these. some grow in early spring in the woods. They appear in the form of cups or wine glasses and produce their spores from the lining of the cup. One very early kind has a beautiful scarlet lining to the cup, which is whitish outside.

*Puff-balls*—They are usually somewhat spherical or pear-shaped and vary in size from less than one inch to a foot or more in diameter. At first white and cheesy inside, they soon become dark colored, changing to a dusty spore-mass when ripe. The Earth Stars or Geasters are small puff-balls in which the outer coating splits into a number of pieces which curl back from the inner part like the rays of a star.

*Gill-Fungi*—These are usually umbrella or fan-shaped fungi, with thin radiating plates or gills on the lower side of the cap or flattened portion. The stem in some species has a ring near the upper end, and in other kinds the stem arises from a sheathing cup-like bulb (volva) at the base. It is among those having this cup around the base of the stem that the deadly poisonous mushrooms occur. The spores are produced on the gills. They are mostly fleshy in consistency and decay readily.

*Pore-Fungi*—The members of this group vary from umbrella-shaped to shelf or bracket-like forms, with minute pores or tubes opening on the lower surface of the expanded part. The spores are produced on the walls of these small tubes. Many of these fungi are hard and woody and do not decay readily. They occur most frequently on decaying wood.

*Spine-Fungi*—These fungi possess about the same forms as the pore-fungi, but the spores form on the surface of soft teeth or spines, which are attached to the expanded portion. Some are much branched and appear on decaying logs and trunks.

*Club-Fungi*—These are mostly low plants, usually on the ground in woods. They appear like little erect clubs, or more often like a tuft of coral-like branches. The spores are produced on the surface of the branches. They are very attractive, especially the bright colored ones.

In collecting specimens for study and preservation, full notes should be made as to date, locality, habitat, collector, and in addition more complete notes will be necessary in some of the perishable kinds. Most fungi are dried for permanent specimens. This should be done as rapidly as possible, especially in the case of the soft kinds.

When dry they may be flattened out by moistening and then pressing gently between blotting paper. The moistening is best done by placing the dry specimens in a box containing a layer of moist sand covered with cloth or thick

paper. Several hours may be needed in the process if the specimens are large.

Gill-fungi should have notes in regard to a number of features which otherwise would be lost by decaying, such as:

1. Color of spores (obtained by cutting off the cap of a specimen and placing it, gills down, on a piece of paper and covering with a tumbler or bell-jar for a few hours. A deposit of the spores will then be found and if placed on gummed paper this "spore print" will be quite permanent).
2. Cap or pileus—dry, moist, viscid?  
Smooth, scaly, shining, striate?  
Color, size, shape.
3. Gills—color when young and when mature?  
Crowded or far apart? Narrow or wide?  
How attached to the stem?
4. Stem—fleshy or tough?  
Solid, with a soft pith, or hollow?  
Size and shape? Color?
5. Ring and volva (a cup-like sheath at base of stem) if present?

A pencil sketch of the fresh plant is also useful, especially one showing a lengthwise section of an entire specimen. This will show the shape of cap, width and attachment of gills, and length and other characters of the stem.

Puff-balls are usually identified from the mature, dry plant. The club-fungi should also be accompanied with notes as to color of fresh plant and spores. The latter may be obtained by covering a plant with a tumbler on a piece of white paper for several hours. The pore-fungi, especially the hard, woody kinds, may be dried at once and identified later.

In sending specimens of the more perishable fleshy fungi for identification, fresh material is always best. Pack the specimens carefully in cotton or slightly moistened sphagnum moss in a stout pasteboard or light wooden box for sending by mail. Do not use tin boxes for fresh specimens, as they decay very quickly under such circumstances. Dried specimens should be pressed out, slightly, and placed in paper folders with the label. Dry puff-balls should be preserved in paper boxes and not pressed. Never press any specimen of fungus perfectly flat, use only enough pressure to straighten them out.

For exhibition nothing can excel fresh specimens of the fleshy kinds of fungi, although puff-balls and the woody kinds dry well and can be used in this condition. The soft kinds, if to be kept for permanent specimens, may be preserved in a mixture of alcohol 1 part, water 2 parts. This, however, is an expensive way of keeping specimens. A mixture of formalin 1 part and water 10–20 parts will preserve the firmer kinds of fleshy fungi fairly well, but does not succeed with the softer ones. Many of the drier kinds of umbrella-shaped fungi may also be dried and exhibited in paper trays or mounted on sheets of heavy white paper by means of narrow strips of gummed paper. They should be accompanied with a spore print and drawings of the fresh plant when possible. Many kinds furnish excellent subjects for water color drawings and photographs.



The following books will be found useful in studying these plants :

The Mushroom Book, Nina Marshall, Doubleday, Page & Co., N. Y. \$3.00.  
(For beginners.)

Mushrooms and Their Use (50c.), C. H. Peck, Cambridge Botanical Supply Co., Cambridge, Mass.

Moulds, Mildews, and Mushrooms, L. M. Underwood. Henry Holt & Co., New York.

One Thousand American Fungi, Chas. McIlvaine. Bowen-Merrill Co., Indianapolis, Ind. \$5.00.

Bulletin 208 Mich. Agricul. College Exp. Sta., Michigan Mushrooms (Morels and Puff-balls), B. O. Longyear. (Free on application to Secy. Mich. Agricul. College.)  
B. O. LONGYEAR.

Michigan Agricultural College.

## Collecting and Preserving Lichens.

The collection and preservation of lichens is a comparatively easy matter. No herbarium insect ever destroys or injures them. Some of the larger, foliaceous forms, like *Usneas*, *Ramalinas*, *Parmelias*, *Peltigeras* and others require a little pressure until dry, so that when placed in pockets they will lie flat. Lichens may be collected at any and all times. Some of the species will be seen anywhere outside the denser part of our cities. No careful search is necessary for the more common sorts, for they grow on the bark of many trees, wooden and stone fences, decaying wood, rocks, and soils. Often as many as twenty species may be found within reach on the bark of a single tree. They are most conspicuous on rainy days. Most people call them mosses. If the lichen is growing on wood or soil a thin-bladed knife will remove it without any of the substance on which it is growing, but often a shaving of bark or soil is necessary to hold the specimen together. This is especially true in case of some of the low forms, which are noticed only as light-colored bands sprinkled with tiny black specks part way around the trunks of young hard maples and red oaks. When the lichen is on rock, if a knife will not remove the lichen without breaking too much, a piece of the rock must be chipped off, or when found on pebbles along the road or elsewhere, the whole thing is collected. Paper bags made to hold two pounds or less are convenient in the field. They can be purchased for a few cents of any groceryman. The time and place of collecting may be indicated on the bag, the material slipped in and the bag closed up and put into the vasculum or basket. After each collecting trip each different bag of material should receive a serial number and this number should accompany every part of that material wherever it goes. This number should be recorded in a book where everything desirable to be known about that material should be recorded. The same species may be under several different numbers. If two species are found under the same number, one should be given a new number and appropriate note made. If the locality, date, collector, number and other proper records are made at the time of collecting, the name of the lichen may be

added years later. At this time the writer has some lichens for determination that were collected in Michigan over fifty years ago. My own record book is bound in half morocco, contains 200 pages with thirty-two lines on a page, with a single vertical red line near the left edge of each page. In the space thus marked off are the serial numbers. On the top line is one number, then on the seventh line below is the next, and so on, leaving six lines under each number for data. Thus, on each page are five numbers, and 1,000 in the whole book. A sufficient amount of material (fruiting material, preferably), to represent the species, is placed in a pocket of tough white paper, made by bringing the bottom of the sheet up to within about one-half inch of the top, and creasing; the top half-inch is folded down and creased, and then about one-half inch of either end is folded back and creased. The shape of the flat sheet will determine the shape of the pocket. Several sizes of pockets should be on hand. The writer has found it most convenient to have labels printed for each locality where much collecting is done, as they can be had for 15 cents per hundred of the Cambridge Botanical Supply Company, Cambridge, Mass. The labels should be pasted onto the half-inch turned down from the top in making the pocket. The pockets should be fastened to herbarium sheets of standard or half-standard size by a dab of liquid glue in the middle of the back of each. They should be fastened around the edge as well as the middle of the mounting sheet, so that when the sheets are placed in a pile it will be of uniform thickness.

A helpful way to learn how to do this is by seeing some prepared specimens. In order to afford this opportunity, and to draw attention to this group of plants, with a view to ultimately finding out what we have in this state, the author has offered to send a set of prepared lichens to high schools of the state that will properly care for them and pay postage—about 24 cents—on same. He will undertake to determine such material as may be submitted. If the specimens are to be returned, postage will be expected of the sender. E. E. BOGUE.

Michigan Agricultural College.

## Directions for Collecting, Preparing, and Preserving Specimens of Orthoptera for the Cabinet.<sup>1</sup>

The writer has been appealed to so frequently for aid in determining collections of Orthoptera for various institutions and individuals in different parts of the country, that it has been decided to make an attempt at building up a typical reference collection of these insects here at the University of Nebraska. In order that we may obtain this material in as excellent a condition as possible, and also to encourage and aid others in their study of the group, the present brief paper has been prepared.

Perhaps no other group among insects has been so much neglected by their devotees as have those belonging to the order Orthoptera. The chief reason

<sup>1</sup> Special Bulletin No. 2 from the Department of Entomology in the University of Nebraska.

for this neglect, perhaps, is the lack on the part of entomologists of the necessary knowledge concerning suitable methods for their preparation and preservation for the cabinet. Their comparatively large size, juicy bodies when alive and just killed, brittleness of limbs and antennæ when dried, their proneness to fading after death, and their liability to the attacks of mould and museum pests, all seem to conspire in a greater or less degree to their neglect. Hence the average entomological student chooses to spend his time in collecting and studying the various other groups which present fewer difficulties that must be overcome in the formation of a collection.

Since a number of these drawbacks named above are more imaginary than real, the writer has thought it worth while to direct the reader's attention to the facts as they are. The large size of most orthopterous insects, instead of being a hindrance to their collection, should rather be considered an advantage, because of aiding in their discovery. Still, if we wish to find small examples of this order we can do so. There are several hundreds of distinct species of such smaller Orthoptera already known to science. The larger and more showy ones have naturally come to light first, while the smaller and less brightly colored forms either remain entirely unknown, or have come to the notice of the very few specialists only who have dared to venture into the almost forsaken field.

Placing these insects in alcohol and other liquid preservatives has in part overcome the objection to their large, soft, juicy bodies that so quickly shrivel and become discolored when treated by the ordinary methods followed for specimens of other orders. But this method of preservation quickly effaces the many bright colors common to such large numbers of them, and even changes minute structural characteristics, so as to render the insects difficult of recognition save by specialists. It also adds to the amount of space required for their reception, as well as a separation of many forms from the general series in the collection. Preserving these insects in spirits also frequently renders it very difficult to transport them on trans-country expeditions in new regions where no roadways occur, both on account of their bulkiness and weight in this condition, and because of the impossibility of always securing the necessary spirits when away from civilization and its influences. True, specimens collected in this manner are always in more or less perfect condition for dissection when this is desired, and for such purposes, when practicable, it is well to make such provision. Again, the objection to injury by *Dermestes*, *Anthrenus*, *Trogoderma* and other museum pests is overcome by spirit-preserved specimens. Still, this latter fault can be remedied in other ways, as will be shown later.

“STUFFING.”—Within the past few years most of the objections that had so frequently been made to the gathering and preservation of orthopterous insects, have practically been removed by the adoption of different and better methods of preparing and preserving these creatures. A few of our specialists only seem to have profited from the discovery that these insects can be handled “taxidermically,” i. e., be stuffed in a similar manner as we would adopt for birds, reptiles and mammals, and thereby preserved in collections equally well with other forms. The following directions for collecting, cleaning and

"stuffing" orthopterous insects may therefore be of much value to those who contemplate making collections of and studying these insects: Instead of throwing the specimens in spirits (alcohol, brandy, whisky, etc.) when captured, they should be killed in the "cyanide" bottle (Fig. 1)<sup>1</sup> from which they should be removed soon after death, and at once opened, cleansed and stuffed; or they can be transferred to a small tin or other box where they may be kept flexible till arrived at home or in camp. Now take the specimens,

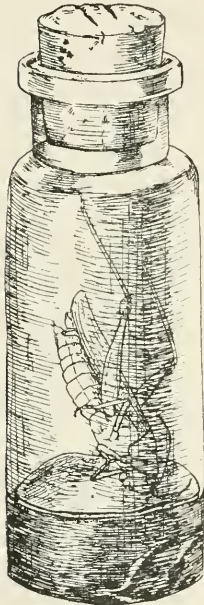


FIG. 1.—Cyanide Bottle.

one at a time, in the left hand, as shown in Fig. 2, and with a fine, sharp-pointed scissors open the abdomen by cutting across the middle of the two basal segments on the lower side, then reverse and cut the opening a trifle larger by nearly severing the third segment. After this has been done, extract all of the insides (intestines, crop, ovaries, etc.), along with the juices, using a fine pointed forceps for the purpose, wipe out the inside of the insect with a small wad of cotton, and it is ready to be "stuffed," or filled up. When this latter is done, the insect may be either pinned into a box prepared for the purpose at once, or it can be wrapped in paper and packed away for future use. To "stuff," cut some cotton bat (raw cotton) in short pieces and fill up the insect through the opening previously made for cleaning it, using the same or a similar pair of forceps for the purpose, taking care not to fill too full nor to stretch the abdomen beyond its original dimensions. When the filling is completed carefully draw the edges of the severed segments together and gently press the sides of abdomen into shape with the fingers. This can all be done, after a little practice, in about four or five minutes' time. The advantages in favor of a

specimen thus handled are several. It will not decay nor turn dark, the original colors will be retained more nearly perfect, and there is but little danger under ordinarily careful treatment of its being attacked in future by the museum pests mentioned. Specimens when thus prepared by an expert and properly labeled are easily worth three or four times as much for cabinet specimens as those not so cared for. Especially is this true with reference to specimens collected in warm, moist climates, where decay is rapid, and where mould is sure to attack specimens that are long in drying.

WRAPPING FOR STORAGE OR TRANSPORTATION.—It is equally important to wrap properly and carefully each specimen in such a manner as to protect all its

<sup>1</sup> The cyanide bottle is made by selecting a suitable wide-mouthed bottle and placing in it a good sized lump of *potassium cyanide*, say about one-half ounce, more or less, according to size of the bottle used. Now mix sufficient good plaster of Paris with water and pour over the lump of potassium cyanide, so as to cover it at least three-fourths of an inch. Allow to stand and harden, then cover with one or two pieces of blotting paper. Close the bottle with a good cork, and your bottle is ready for use. When moisture gathers inside, wipe out and renew the blotters. Be careful not to inhale the fumes of the cyanide, for they are poisonous. In place of the plaster the cyanide may be covered with crumpled paper and held in place by a tight-fitting card-board that has been previously punctured with a pin to let through the poison fumes.



parts, as well as its original shape, in order that when received by the museum authorities or the private collector it can readily be transferred to its proper place in the collection as nearly perfect as possible. For this purpose cylinders or tubes of porous paper are best. These can be made by rolling the paper over small cylinders of light wood of various diameters as indicated in the illustration numbered Fig. 3. When rolled, fasten the paper with a little paste, slide partly off the wood and fold shut one end. Now shove a little cotton into the tube until it rests on the folded end, fold the legs and antennæ of your specimen to its body and push into the tube head-first. Put in a second small wad of the cotton and fold shut the other end, taking care not to twist or injure the tips of wings, legs or other parts of inclosed specimen. To insure this it is necessary to have the paper tubes plenty long to allow for folding the ends. When placed in papers, as just described, the specimens need still further care, according to locality and climate in which collected. If

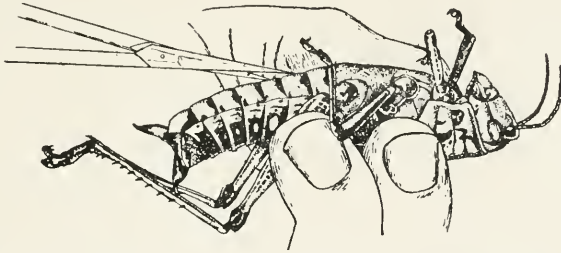


FIG. 2.—Showing method of opening orthopterous insect for “stuffing.”

in a desert or other arid region, if simply packed loosely in a light wooden or other box, so as to allow for a free circulation of air for drying, will suffice. Even non-stuffed specimens of small and moderate sized insects, when thus wrapped and allowed to dry quickly, will make fair cabinet specimens. In moist, warm regions, however, they must be dried artificially in small portable or other ovens, and then packed in tin boxes along with a little carbolic acid or naphtha crystals for destroying mould and keeping out insect pests that otherwise would soon destroy them. Flat-bodied forms, like the cockroaches (*Blattoidea*), especially the larger kinds, can best be wrapped in small paper envelopes or triangles instead of tubes.

**CLEANING MOULDY SPECIMENS.**—If perchance specimens of considerable value should become mouldy, or such should be received from persons who are not familiar with the best methods of handling these insects, they can usually be saved. Although not making the best of cabinet specimens, they will nevertheless fairly well represent the species until replaced by better. These mouldy specimens should be placed in a tin box between wet cloths or blotting papers that have been pretty well sprinkled with dilute carbolic acid and leave for twenty-four to thirty-six hours or until sufficiently soft not to break when handled. Then pour some alcohol into a dish and add to it about one-twentieth as much liquid carbolic acid. With a camel hair brush carefully clean the entire

insect, taking care to wash every portion with the mixture of alcohol and acid. This same method can be followed in destroying the mould on cabinet specimens.

PINNING AND ARRANGING FOR THE CABINET.—Here we may also adopt such methods as will materially lessen the number of objections that have so frequently been urged against Orthoptera as suitable specimens for the average student of entomology to occupy himself with. Having shown in the preceding paragraphs that loss of color, form, the injury by museum pests, etc., can be avoided by a little proper care in collecting and handling them, the most important matter that now remains for us to do in

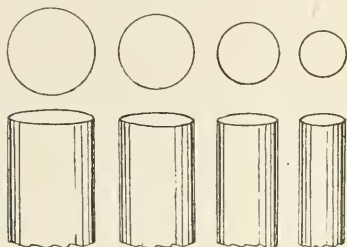


FIG. 3. —Representing wood cylinders ( $\frac{3}{4}$  actual size) for making paper tubes in which to pack orthopterous insects.

pinning and arranging the specimens for the collection is the economizing of space and preserving a neat appearance in the specimens themselves. In doing this we must keep in mind the characters that must be used in studying and classifying the insects under consideration. We must also aim to preserve the antennæ and legs intact. In the winged forms a single specimen can be made to show the insect with these appendages both spread and folded. (See Fig. 4, *b*.) The antennæ, when long, should be directed backwards along

the sides of the body of the insect. In like manner, the legs can be so folded and crossed as to take up the least possible amount of room and still be accessible for the student's use in studying the specimen, as is shown at *c* in Fig. 4, a "walking-stick." A single specimen, in this manner, can be made to

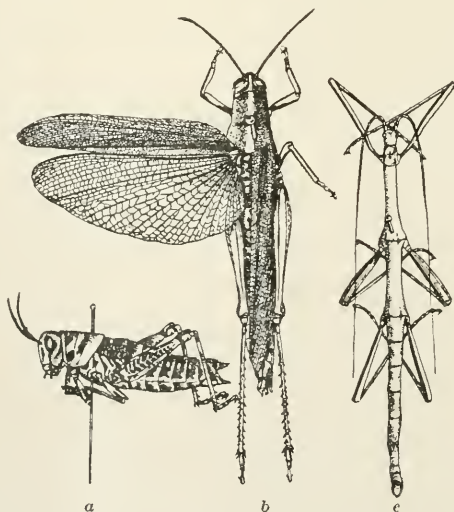


FIG. 4.—*a*, *Dactylotum pictum*; *Schistocerca emarginatum*; *Diapheromera femorata*—showing manner of pinning.

show all that is necessary for the study of that sex of the species to which it may chance to belong.

When pinning, care should be taken to so locate and pass the pin as to hold solidly the insect, and at the same time not interfere in studying it afterwards. In the Saltatoria, or jumping forms, a correct method is to insert the pin near the back edge of the pronotum, a little to one side of the middle, and directing it to the rear, letting it pass downward through the mesothorax, thereby tightly fastening together the two sections of the body. (See Fig. 4, *a*.) In the other forms (*Blattoidea*, *Mantoidea* and *Phasmoidea*) the pin should be inserted back of the pronotum and through the middle of the body, taking care to select a solid portion for this purpose, without running the pin through the basal portion of any of the legs.

In pinning these insects directly from the cyanide bottle they can be arranged so as to fit closely in the box. This is done by sticking the pins into the lining so as to allow the tips of the insects' bodies to nearly rest on the bottom of the box or cork lining. The next and following rows can then partly overlap the ones just preceding. This method of pinning also allows the hind legs to rest upon the bottom of the box and keeps them from jarring loose, as well as putting them in place for the future.

#### LIMITATION OF THE ORDER ORTHOPTERA.

This order, as now restricted, is made up of six super-families, as follows: *Blattoidea*, *Phasmoidea*, *Mantoidea*, *Acridioidea*, *Lacustioidea*, and *Grylloidea*. The members of the first are known popularly as "cockroaches;" of the second, as "walking-sticks," "spectres," "leaf-insects," etc.; of the third, as "mantides," "praying insects," "rear-horses," etc.; of the fourth, as "locusts," or "short-horned grasshoppers;" of the fifth, as "long-horned grasshoppers," "katydids," "stone-cricketts," "camel-cricketts," etc.; and of the sixth, as "cricketts," "tree-cricketts," "mole cricketts," etc. All of these forms are directly interesting to the economic entomologist on account of their food habits, while many of them are intensely so on account of their mimicking forms which show striking resemblances to portions of various vegetable organisms.

LAWRENCE BRUNER.

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The following description of a simple and inexpensive apparatus for collecting atmospheric dust may be of some interest to bacteriologists and amateur microscopists: The apparatus consists essentially of a wire, the middle portion of which is coiled into a spiral to fit over and turn on a vertical support. One end of the wire is bent so as to hold a strip of glass, and the other end is bent so as to clamp a piece of cardboard, serving the purpose of keeping the plane of the glass at right angles to the direction of the wind. The support comprises a tenpenny nail driven into a block of lead. Soldered upon the head of the driven nail is the head of a second nail. The heads thus placed in juxtaposition serve as a shoulder upon which the coil of wire rests.

The glass plate is smeared with glycerine upon which the dust adheres. The apparatus can be set in any convenient place where the wind blows, and the plate examined from time to time.—*Sci. Am.*

## A Review of the Methods of Staining Blood.

### IX.

#### D. *Neutral Stains*.—Continued.

*Goldhorn* (1901), who has obtained neutral staining of the blood by successive staining with eosin and his polychrome methylen blue, has since combined the two into a neutral mixture and isolated the neutral dye. This he does as follows: To his polychrome methylen blue (III D, 5, i), made strongly acid with glacial acetic acid, is added 5 per cent. eosin solution till the mixture is transformed into a pulpy mass. This is filtered through two layers of filter paper, and the mass left on the paper is dried in a hot-air oven, after which it is dissolved in wood alcohol. If the solution thus prepared is too acid, staining the erythrocytes too deeply and the leucocytes very little or not at all, the desired reaction may be obtained by adding gradually wood alcohol, made alkaline with potassium carbonate, until the dye gives the required results. If the stain is made strictly neutral or alkaline, the red corpuscles will stain a greenish hue and the granules and "anaemic degenerations" will be shown while they will not be seen when the dye is acid.

To use the stain the preparation is flooded with the dye for a few seconds and then washed with water. Acidophile granules are differentiated by allowing the stain to act for about 15 seconds. The strength of the stain may be altered by varying the amount of alcohol. The red corpuscles stain pink, eosinophile granules more or less red, nuclei of leucocytes from blue to purple, and granules of the mast cells most prominently metachromatic, malarial parasites as by Plehn's solution, the nucleus of the young form being well shown. Blood platelets are also stained.

*Giemsa* (1902) considers the neutral precipitate from the eosin and methylen blue mixture isolated by Reuter to consist of eosin salts of methylen blue, methyl violet and azure, the two latter being impurities of the methylen blue. He has prepared a stain of eosin and pure azure which he says gives a clear and sharp staining with all the differentiations of the Romanowsky-Nocht stain. His formula is as follows:

0.05 per cent. solution of eosin (Höcht),	-	-	19 c. c.
0.8 per cent. solution of azure,	-	-	1 c. c.

Stain the preparations a few minutes and then wash them with water.

*Wright* (1902) has modified Leishman's method. His staining solution is prepared as follows: To a  $\frac{1}{2}$  per cent. solution of sodium bicarbonate in water add 1 per cent. of methylen blue (Grübler). This mixture is steamed in an Arnold steam sterilizer for one hour, and when cool one to one thousand solution of eosin (Grübler, yellowish, soluble in water) is added until the mixture, losing its blue color, becomes purple in color and a scum with yellowish metallic luster forms on the surface, while, on close inspection, a fine-granular black precipitate appears in suspension. (This requires about 500 c. c. of the eosin solution for



100 c.c. of the alkaline methylen blue solution.) This precipitate is collected on a filter and without washing is allowed to dry. When thoroughly dry a saturated solution is made in pure methylic alcohol. (Three-fourths of a grain of the dry percipitate will thoroughly saturate 100 c. c. of the methylic alcohol in a few minutes.) This saturated alcoholic solution of the percipitate is next filtered and 25 per cent. of methylic alcohol added to the filtrate. This somewhat dilute alcoholic solution of the percipitate is the staining fluid. It is permanent and may be kept on hand ready for use, but care should be taken to prevent evaporation of the alcohol, thus making the solution too concentrated.

Wright's staining technique is as follows :

1. Make films of the blood, spread thinly and allow them to dry in the air.
2. Cover the preparations with the alcoholic solution of the dye for one minute.
3. Add to the alcoholic solution of the dye on the preparation sufficient water, drop by drop, until the mixture becomes semi-translucent, and a yellowish metallic scum forms on the surface. Allow this mixture to remain on the preparation for two or three minutes.
4. Wash in water, preferably in distilled water, until the film has a yellowish or pinkish tint in its thinner or better spread portions.
5. Dry between filter paper and mount in balsam.

The blood film is fixed upon the cover-glass by the action of the concentrated alcoholic solution for 1 minute before the dilution. The real staining takes place after the dilution with water.

The red corpuscles are stained orange or pink, nuclei some shade of blue, acidophile granules eosin color, neutrophile granules reddish lilac, basophile granules of the mast cells dark blue or dark purple, blood plates blue or purplish. The bodies of malarial parasites stain blue, while the color of the chromatin varies from a lilac through varying shades of red to almost black.

Massachusetts State Board of Health.

ERNEST L. WALKER.

## The Museum.

### VII.

#### THE HALL.—Continued.

The floors of halls, as previously mentioned, should be of hard, easily washed substances. Tiling meets these requirements. Tiling is expensive, and it may be necessary in some cases to resort to a substitute. Wood wears away too easily, but protection to it can be secured by staining and rubbing its surface with a dressing of linseed oil and turpentine, with some admixture of crude oil.

In this connection it seems altogether feasible to use an asphalt or concrete floor, colored with red or yellow ochre. This can be made durable and hard, is much cheaper than tiling, and admits freely of washing, and, carefully laid down, would probably serve most usefully.

The height of ceilings in museum halls and the ceilings themselves, their

method of support or construction, are vexed questions. At the outset it is quite evident that the height of ceilings should have a relation to the width of a hall, as the wider the hall the more necessary to have high ceilings for the penetra-



FIG. 28.—Chicago Academy of Sciences.

tion of light across it from *high* windows. It is also clear that the ceilings of all halls on one floor should have the same height. A good hard and fast rule



FIG. 29.—Delivery Hall, Public Library, Chicago.

is, to make the height of a ceiling one-third the width of the hall. This will generally admit of satisfactory illumination, if light is admitted from both sides, and tolerable illumination, if admitted from only one. These are architectural

exigencies which frequently require varying heights on different stories, and as generally, with the same width, the top or attic story is much reduced in height, it is best to eliminate this floor, if possible, from the exhibition spaces, unless top-lighted, or the architect will permit a proper spaced ceiling to be given to it. It is, however, possible to produce an architectural effect of much dignity by making the top-story high-ceilinged (?), as is seen in the completed wing of the Chicago Academy of Sciences (Fig. 28).

In regard to the construction of ceilings in wide halls it is generally indispensable to use columns, and it is the writer's opinion that while adding conspicuously to the beauty of a hall they can be so placed as not to limit its space or prove an interference to cases. On the other hand, as in the large mineral hall of the American Museum, huge steel girders can span the entire width of a sixty foot hall, and all columns can be banished. And thirdly the construction can often, in favorable positions, be domed when columns and girders are equally eliminated, as is seen in the beautiful delivery hall of the Public Library at Chicago (Fig. 29).

L. P. GRATACAP.

American Museum of Natural History.

## Bacteriology for High Schools.

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### III.

#### MICROSCOPICAL EXAMINATION OF BACTERIA.

The bacteria which have developed on the potato cultures should be examined under the microscope so that the individuals may be seen and studied.

There are two ways of doing this. They may be examined in a living (or unstained) condition, or they may be examined after they have been stained. Each method has its distinct advantages, and both are always used in the systematic study of any micro-organism. The latter is the least difficult and will be described first.

*Preparation of Stain.* The anilin dyes are used, and for ordinary puposes fuchsin (magenta) methylen blue or gentian violet may be used. These dyes may be purchased in a dry form, or as saturated alcoholic solutions; the latter is the most convenient form. The alcoholic solution is not suited for use, but must be diluted with water. This may be done by adding to nine parts of water, distilled, or at least boiled, one part of the saturated alcoholic solution of the stain. Or the solution may be made by placing water in a test tube or vial one-half inch in diameter, and then adding to this, little by little, the saturated alcoholic solution of the stain until it is just opaque.

*Examination of Stained Bacteria.* On a glass slide which has been well cleaned, place a small drop of recently boiled water. With a platinum needle or hat pin, which has been heated and allowed to cool, touch one of the colonies on the potato cultures. The needle, or pin, is now introduced into the drop of

water on the slide, and with the needle the water is spread over the surface of the slide, covering a space about one-half inch in diameter.

The slide is allowed to remain until the film is thoroughly dry. Holding the slide by one end, film side up, pass it through the upper part of the flame of a Bunsen burner, or of an alcohol lamp; each passage should consume about one second of time. This is for the purpose of fixing the bacteria to the slide, but where a flame is not at hand this may be omitted, although under these conditions some of the bacteria may come off in the subsequent washing.

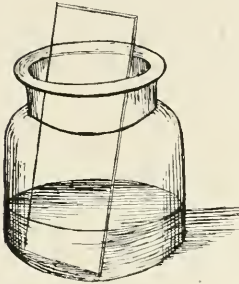


FIG. 11.—Wide mouthed bottle filled with stain, for use in staining bacteria on glass slide.

A small, wide mouthed bottle is filled with the staining solution, and the slide, with the dried film, is placed in the stain (see Fig. 11), and allowed to remain from five to ten minutes. It is then removed and the excess of dye washed off. The washing should be continued until the water is no longer colored by the dye. A clean cover glass is now to be placed over the film. One edge of the cover glass is placed in contact with the slide, and then gently lowered into place.

The excess of water is removed from the slide and cover glass with filter or blotting paper. The preparation is now ready to be examined under the microscope. Use the highest power obtainable, an oil immersion lens if possible.

#### Résumé :

1. Spread the film on slide.
2. Dry in air.
3. Fix by passing through the flame.
4. Stain.
5. Wash in water.
6. Cover with cover glass.
7. Examine.

In examining these the maximum amount of light is needed.



FIG. 12.—Diagram illustrating cell grouping among the Micrococci. 1, Micrococcus (Staphylococcus); 2, Diplococcus; 3, Streptococcus; 4, Tetrads; 5, Sarcina.

Make preparations in this way from colonies which present varied appearances on the potato cultures, for illustrations of the various form types.

On the piece of potato exposed to the air will probably be found colonies of various micrococci, or spherical bacteria. The different forms will vary in size and in the grouping of the cells. When spherical bacteria are isolated or arranged in irregular groups they are spoken of as *Micrococci*, sometimes *Staphylococci* (Fig. 12,<sup>1</sup>). When they are in pairs they are called *Diplococci* (Fig. 12,<sup>2</sup>),



in chains *Streptococci* (Fig. 12,<sup>3</sup>), in fours *Tetracocci* (Fig. 12,<sup>4</sup>), and in groups of eight, sixteen, etc., i. e., growing in three dimensions producing packets, they are termed *Sarcinae* (Fig. 12,<sup>5</sup>).

The piece of potato inoculated with dirt will undoubtedly show colonies of rod shaped bacteria. Some of these may be narrow with pointed ends (Fig.

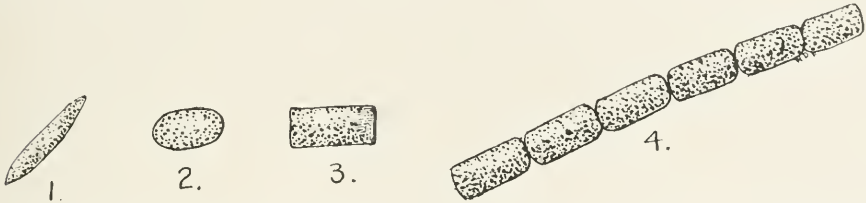


FIG. 13.—Diagram illustrating form and cell grouping among the rod shaped bacteria.

13,<sup>1</sup>), others shorter with rounded ends (Fig. 13,<sup>2</sup>), others have square ends (Fig. 13,<sup>3</sup>). Not infrequently the rod shaped bacteria remain attached, end to end, forming threads (Fig. 13,<sup>4</sup>). The spiral forms are not likely to be represented on the potato cultures. They are sometimes found in the tartar from



FIG. 14.—Diagram illustrating form and cell grouping among the spiral shaped bacteria. 1, *Microspira*; 2, *Spirillum*; 3, *Spirochaeta*.

the teeth, and may also be found in water from a bouquet, or in a hay infusion. This is easily prepared as follows: Place a small handful of hay or straw in a barrel, cover it with water and place in a warm place for a day or two.

The three typical forms are the slightly bent rod, or *Microspira* (Fig. 14,<sup>1</sup>), the double bent rod, or *Spirillum* (Fig. 14,<sup>2</sup>), and the long spiral form, or *Spirochaeta* (Fig. 14,<sup>3</sup>).

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W. D. FROST,  
E. G. HASTINGS.

The Lake Laboratory of the University of Montana will open on July 13 and will continue for five weeks, after which opportunity will be given for research work. The field laboratory is located on the bank of Swan river at its outlet into Flathead lake. This location affords a harbor for boats and a camping site for the tents of those attending. The adjacent region contains forests, ponds, lakes, swamps, cultivated fields, mountains, rivers, and ravines. It is rich in animal and vegetable life. The lake offers opportunities for collecting, and presents some beautiful scenery. East of the lake the Mission range comes abruptly to the water's edge. West of the lake are the Cabinets. Near the station Swan lake, Rost lake, Echo lake, and other waters, are easily accessible. *Daphnia* pond, a few minutes' walk from the station, is rich in pond life, while Estey's pond, about as far again, is fully as productive. The Swan range is easily accessible from the station, and Alpine summits are annually visited. The station is not difficult of access. The stage and boat rides are easy, with charming scenery constantly in view. The building is a convenient out-door laboratory, with tables for a dozen students. The station work has entirely outgrown the building. Many of the lectures are given out of doors in the yard, and the fine summer weather permits of much laboratory work out of doors. Information may be obtained from Prof. Morton J. Elrod, director of the station, University of Montana, Missoula, Mont.

## Methods in Plant Physiology.

## XII.

## HELIOTROPISM—Continued.

5. **Localization of the Sensitive Area.**—This experiment is designed to answer the question, Are the organs of the reception of the stimulus and the organs of response the same tissue? Raise in the dark chamber seedlings of German millet (*Setaria Italica*) in a crock filled with earth level with the rim. While germinating, this culture must be attended every day to insure having the proper supply of moisture present. When the plants have reached a height of 1 cm. above the earth, caps of tin-foil are to be slipped over three or four of them. These caps are made by rolling pieces of tin foil 5 mm. by 10 mm. around a pin whose head has been cut off. Thus a cylinder 10 mm. long is made. One end of the cylinder is to be flattened and turned at right angles before removing it

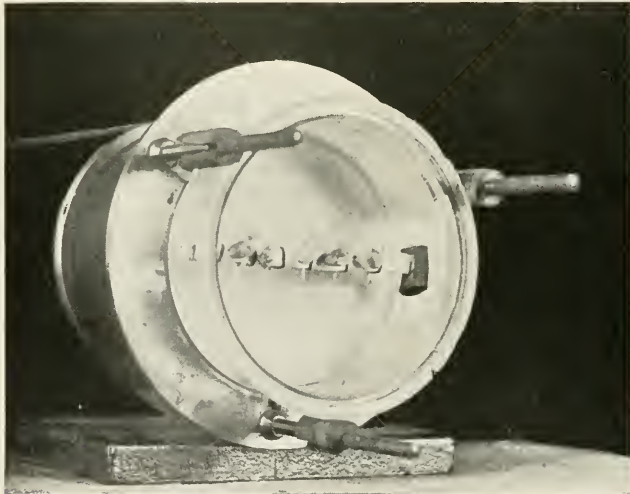


FIG. 13.—A damp chamber in use on a klinostat. The power is furnished by a small electric motor. The speed is reduced by the intervention of a worm-gear.

from the pin, the flattened part being about 3 mm. in length. The caps should be handled by means of small forceps only. At the expiration of 6 to 12 hours the contrast between the capped seedlings, which remain upright, and the others, which are bent strongly towards the light, is very marked. Proving that the sensor and motor organs are in different tissue.

6. **Growth of Stems and Roots when Light is Excluded and Gravitation is Neutralized.**—Employ seedlings of corn (*Zea Mais*) 4 to 6 cm. long and fasten them to supports in a damp chamber as shown in Fig. 17. The damp chamber should be immediately started revolving on a klinostat in a dark room. Some of the seedlings should be placed parallel and others at right angles to the axis of revo-

lution. Should the long axis of the seedling make less than a right angle with the axis of revolution, *gravitation is not wholly neutralized*. Observe the direction of growth in both stems and roots at the end of 8 to 12 hours. If properly set up they will continue to grow in a straight line.

7. **The Directive Action of Gravitation when Light is Excluded or Neutralized.**—The first condition is fulfilled by placing a plant horizontally in a dark chamber, and is practically the same as the first experiment under the study of Geotropism.

To fulfill the second condition, change the damp chamber of the klinostat from the vertical position shown in Fig. 17 to a horizontal position, and fasten several mustard (*Sinapis alba*) seedlings to the wooden bar in such a manner that they will be in a horizontal position when the bar is inserted in the damp chamber. Cover the damp chamber of the klinostat with a dark box which has a small window admitting light on a level with the horizontally revolving seedlings. In both cases it will be found that pronounced geotropic curves appear.

8. **The Directive Action of Light when Gravitation is Neutralized.**—Cover the damp chamber of the klinostat with a light proof jacket, leaving an opening of 1 square cm. on one side of the chamber. The covering may easily be made of black paper and held in place with rubber bands. Adjust the damp chamber in a vertical plane as in Fig. 17, so that gravitation will act equally on all sides and light comes constantly to the plants from one side. Mustard or radish (*Raphanus sativus*) seedlings should be used in this experiment. If adjusted properly, they will exhibit heliotropic curves at the end of 8 to 10 hours of revolution.

9. **Direction of Growth when the Stimuli of both Light and Gravitation are Neutralized.**—Employing the same apparatus used in the last experiment, remove the dark covering from the damp chamber of the klinostat and cover the klinostat with a dark box containing a small window, in front of which the seedlings pass as the chamber revolves. As before, use mustard or radish seedlings. The result will perhaps be a little more striking if the seedlings are first laid in a horizontal position long enough to acquire decided curves. The curves will be greatly diminished or straightened out entirely after 12 to 20 hours of revolution.

University of Michigan.

HOWARD S. REED.

## Laboratory Outlines for the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

### THE HIGHER FUNGI AND LICHENS—Continued.

XXXIX. *Bovista plumbea* Pers. Class, Basidiomycetes. Order, Lycoperdales. Family, Lycoperdaceæ.

This puffball of a dark-brown color, when mature, is usually abundant in pastures, where it may be gathered in any season. It has a more or less spherical body, usually from one-half to one inch in diameter.

1. Sketch one of the fruiting bodies, showing the inner peridium with an aperture at the apex for the discharge of the spores.

2. Pick out some of the internal mycelium (capillitium) and after moistening with alcohol mount in water. Under high power draw some of the dichotomously branched mycelium and some of the spores. Describe. How does this plant obtain its nourishment?

XL. *Lichenes*. Ascolichenes.

Lichens grow on the bark of trees, on wooden fences, on rocks and on the ground. The common forms may be collected and kept indefinitely in a dry condition in wooden or paper boxes. Lichens are associations of fungi and algæ. They represent a condition of symbiosis known as helotism, i. e., the fungus is a slaveholder, the algæ are slaves.

(a) *Parmelia caperata* (L.) Ach.

This lichen is of a light green color and is very abundant on trees and fence boards and rails, forming large circular thalli often a number of inches in diameter.

1. Study the naked eye characters of the thallus. Draw a part of the thallus, showing the margin.

2. Soak the thallus in water and tease out a small piece on the slide with needles. Study under high power. Notice two kinds of cells, colorless septate hyphæ, the lichen fungus, and green spherical cells, the lichen algæ. Draw a piece of the mycelium and some of the algæ. To what group do the algæ belong? How are the algæ and the fungus hyphæ arranged in the lichen thallus?

3. Draw two or three algæ, showing the manner in which the fungus grows around the green cell to obtain its food.

4. Vegetative propagation. The alga and the fungus each reproduces itself in the manner peculiar to its species, but the lichen may also propagate itself directly by means of little granular flakes produced on the upper surface of the lichen thallus, known as soredia. Mount some of the granular material in water and examine under low power; notice in favorable specimens that the fungus and algæ are both present in the soredium. Draw and describe.

(b) *Sticta amplissima* (Scop.) Mass.

This is a foliaceous lichen of a light gray color which grows on the bark of trees in forests.

1. Soak the thallus in water and note the change in color of the upper surface. Make a sketch showing the position of the brown disc-shaped or cup-shaped apothecia.

2. With a razor, cut free hand cross section of a piece of the thallus containing an apothecium. Hold the piece between two strips of dry elder pith. Mount the sections in water and under low power draw, showing the green algal layer, the white layer and the position of the apothecium.

3. Under high power study the hymenial layer of the apothecium. Draw one of the asci containing spores. Describe. How many spores? Draw a single spore. Draw one of the paraphyses.

(c) *Endocarpon miniatum* (L.) Schaer.

This lichen with a rather leathery thallus is common on limestone, where it may be obtained at any time of the year.

1. Lay the thallus on the slide without a cover-glass and examine under low power. Draw a part of the thallus, showing the pores which open into the perithecia below.

2. Cut cross-sections of the thallus and mount in water. Sketch under low power, showing the algal and fungal layers and the perithecia.

3. Draw one of the perithecia under high power; also an ascus containing spores.

JOHN H. SCHAFFNER.



## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

**Mottier, D. M.** The behavior of the chromosomes in the spore mother-cells of higher plants and the homology of the pollen and embryo-sac mother-cells. *Botanical Gazette*, 35: 252-282, pls. 11-14, 1903.

*Lilium Martagon* and also the corresponding divisions in the embryo-sac mother-cell of *Lilium Martagon*. Prof. Mottier confirms the accounts of those who have described two longitudinal splittings of the chromosome at the first division of the mother-cell. The first fission occurs in the early prophase and the second during the metaphase or anaphase. The second fission seems to be a preparation for the second mitosis. After the first mitosis, the chromatin of the daughter nuclei becomes reticulated and almost reaches the structure of the resting condition, so that all trace of individual chromosomes is lost. At the second mitosis the chromatin spirem does not split longitudinally, the splitting becoming evident only after metaphase has been reached. There is nothing to indicate that one chromosome differs qualitatively from another. The shape of the chromosomes is of secondary importance and is determined by the shape of the daughter segments and the manner in which the spindle fibers are attached. The chromosomes seem to be oriented in the nuclear plate by a pulling and pushing action of the fibers.

The microspore and megaspore mother-cells are homologous. The row of four megaspores is regarded as the primitive type while the single megaspore, as in *Lilium*, is a derived condition.

C. J. C.

**Molisch, Hans.** Amœben als Parasiten in Volvox. *Ber. d. deutsch. Bot. Gesell.*, 21: 20-23, pl. 3, 1903.

Prof. Molisch reports as a new discovery the presence of amœbæ in *Volvox*.

He has evidently overlooked the work of Hicks, published in 1860, in which a very complete description of "amœboid bodies" in *Volvox* is given.

According to Molisch the amœba attacks the cœnobium from without by thrusting in a pseudopodium. The number in each *Volvox* colony varies from ten to thirty. He could not determine whether all the amœbæ entered from without. As they were only found late in November, Molisch concludes that, as the vegetative period nears an end, the *Volvox* cells become less resistant to attacks of the parasite.

The account given by Hicks does not differ from that of Molisch except in interpretation. Hicks did not believe that an amœba could successfully attack a living cell, and as he found for each amœba present, a cell of the cœnobium absent and the amœba usually occupying the place of the missing cell, he concluded that the cell was transformed into an amœba.

A careful examination of the literature of a subject often changes a discovery into a confirmatory account.

W. J. G. LAND.

Chicago.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

**Schoenemann, A.** Färbung und Aufbewahrung von Schnittserien auf Papierunterlage. Zeitschr. f. wiss. Mikros. u. f. mikr. Tech. 19: 150-161, 1902.

The author encountered a need for good methods in embedding and sectioning large objects, to be kept afterwards.

The author tried plans to see whether it would be possible to fasten unstained microscopical celloidin or paraffin sections direct on a flexible suitable stain-proof paper. The following process resulted from his efforts:

Sections were desired of the nasal cavity of infants and the petrous bone of adults. These were decalcified in 7 per cent. sulphuric acid, or saturate solution of sulphurous acid. As long as the material remained in the acid it was hard, but on removal to water the altered salts were dissolved. The objects were then put in absolute alcohol for an hour or a day, according to the size, and transferred to a mixture of ether 2 parts, oil of cloves 1 part, then into Stepanous collodion solution (celloidin chips 1.5 grm., clove oil 5 grms., ether 20 grms., absolute alcohol 1 grm., added by drops). Small objects were left in this for a couple of hours, larger ones for the same number of days. When infiltration is complete the objects are oriented in paper boxes on a sheet of glass and embedded, hardened for twenty-four hours in chloroform, then the paper is removed and the objects again put into chloroform. If the wet method is used chloroform is omitted and the blocks hardened in 80 per cent. alcohol, but the author prefers the dry method, sticking the block on with thick collodion or paraffin and using the knife at an angle of about 40°. Strips of tracing paper which has been so treated with minerals as to be incapable of being stained is used, one end being kept for notes, etc.; on the other are laid in order the cut sections, which are later dried, flattened with filter paper; there is no danger of their sticking to the filter paper. These strips of paper, in their proper order, are put into 80 per cent. alcohol, where the cedar oil is removed. It is important that the strips should be piled on each other with a filter paper strip between each, and a glass on the top to keep them flat during the hardening process. In this liquid with several changes the sections may remain unhurt a long time. For staining, the strips are put in water, and if cloudy spots appear it is of no consequence. The sections are then stained (dilute hemalum, Gruebler's, or dilute Grenacher's or Delafield's), for several hours, to make the nuclei clear. When sufficiently stained, sections are put in tap-water and put in a solution of eosin in 95 per cent. alcohol. From this they are put into carbolxylol (1-3), in which all cloudiness and any excess of eosin disappears. Then a bath of xylol is used to remove the carboic acid, which would injure the staining properties of the tissue. If they are to be studied wet the strips must now be put in cedar oil, where they may be kept for some months without injury, and examined in cedar oil on a slide covered with glass or mica. If they are to be dried, they are coated with a varnish that will not become brittle and is free from alcohol and ether. "Elaslinlack" (Grübler) is satisfactory; the paper strips lie in this mixture for about twenty-four hours and are then fastened on a wooden frame. They are dry in from twelve to twenty-four hours; during the drying the strips must be brushed over several times, to be sure the sections are well covered, otherwise they may flake off. When after two to six days the strips are no longer sticky, they are laid between filter papers in a cool place.

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

**Bürger, Otto.** Weitere Beiträge zur Entwicklungsgeschichte der Hirndineen. Zur Embryologie von *Clepsine*. Zeitschr. f. wiss. Zool. 72: 525-544, Taf. 30-32, 1902.

in this research in preference to the less favorable ones of *C. bioculata*. Eggs and young embryos were fixed in dilute Flemming's fluid. The older embryos abandoned by the mother or already free-swimming were also killed in hot sublimate or in 10 per cent. nitric acid with excellent results. Elements of the germ bands are clearly demonstrated in sections of embryos gilded by Löwit's method. Heidenhain's iron hæmatoxylin with after-stain of eosin for older embryos was used in staining.

C. A. K.

**Marcinowski, K.** Das untere Schlundganglion von *Distoma hepaticum*. Jenais. Zeitsch., N. F. Bd. 30: 544-550, Taf. 27, 1903.

fibre tracts and Delafield's or Grenacher's hæmatoxylin followed by picric acid for ganglion cells, and van Gieson's method for the nervous system as a whole.

C. A. K.

**Perkins, H. F.** The Development of *Gonionema murbachii*. Proc. Acad. Nat. Sci. Phila., 1902; 750-790, pls. 31-34, 1903.

and spawning may be artificially induced by keeping the medusæ in the dark for an hour, especially in the afternoon. The eggs are adhesive, and if allowed to settle on glass slides they adhere so firmly that they do not wash off when treated with reagents. It is possible to gather them in masses by stirring the water in which they have been deposited, so that they gather in clusters and adhere to each other instead of the bottom of the dish. Corrosive-acetic and full strength formalin were used as fixing agents. The latter gave excellent results both for fixation and preservation, and fixes tissues in fifteen to forty seconds. Menthol crystals were used for narcotising larvæ and adult medusæ. Larvæ were kept alive for six months in balanced aquaria in the laboratory without change of water. Fresh water was added to make good the loss by evaporation from time to time. Cultures of diatoms made from scrapings from eelgrass were added to furnish the required plant life.

C. A. K.

**Penard, E.** Notice sur les Rhizopodes der Spitzberg. Arch. f. Protistenkunde, 2: 238-282, mit 49 Textfiguren, 1903.

Amsterdam Island and Green Harbor, collected for botanical purposes. The dried mosses were placed in pure fresh water with all precautions to prevent contamination by foreign species, and the reviving *Rhizopoda* were studied as they appeared. One cluster of moss dried and revived three times in succession gave the same assemblage of organisms at each revival.

C. A. K.

On account of the relatively large embryos and the greater size of the cell elements of the germ bands, the eggs of *Clepsine sexoculata* were used

The author recommends von Rath's and Mallory's hæmatoxylin (on sublimate material) for demonstration of

Material was collected in the eel-pond at Wood's Hole in summer months.

Eggs are deposited regularly at dusk

The material upon which this study was made was secured from clusters of moss, principally *Hypnum*, from

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory,  
University of Michigan, Ann Arbor, Mich.

**Wallengren, H.** Zur Kenntniss der Galvanotaxis. II. Eine Analyse der Galvanotaxis bei Spirostomum. Zeitschr. f. Allgem. Physiol. 2: 516-555, 1903.

As the result of a much more thorough study than has ever before been given to the electrotaxis of Spirostomum, the author comes to the conclusion that the well known phenomenon of transverse orientation to a constant electric current which this infusorian exhibits is to be explained on the same general lines as the kathodic electrotaxis of the other ciliates. The reason that Spirostomum remains in the transverse position while a current of medium intensity passes, is, according to Wallengren, that in this position the cilia on the kathode side of the body develop approximately as much energy in the expansive phase of their beat, as do the cilia on the anode side in their contractile phase. Hence there is no further movement.

R. P.

**Korentschewsky, W.** Vergleichende pharmakologische Untersuchungen über die Wirkung von Giften auf einzellige Organismen. Arch. f. exper. Path. u. Pharmacol. 49: 7-31, 1902.

The author's purpose in making this study was to get light, by comparative methods, on the pharmacological action of a number of important drugs. The experiments were performed on a number of common infusoria, including Paramecium, Vorticella, Spirostomum, Stylonichia, Euplotes and others. The method used in experimentation was to mix together, by means of two fine tubes of the same caliber, equal quantities of the solution to be tested and of the culture water containing the organisms. The mixture was then put on a glass slide, in a moist chamber, and the changes in the infusoria observed with a microscope. All of the large number of drugs tested fell into two main groups on the basis of their action on infusoria. The substances in the first of these groups (examples: salicylic acid, sodium salicylate, benzoic acid, bromine and iodine salts, morphine, cocaine, etc.) paralyze all parts of the excretory apparatus of the infusoria. The substances in the second group (examples: caffeine, theobromin sodium salicylate, strophanthin, strychnine nitrate, veratrin chloride, physostigmin salicylate, etc.) cause the products of metabolism to be immediately separated out of the protoplasm. The drugs in the first of these groups are substances which tend to produce phenomena of depression (particularly of the nervous system) in higher organisms. The substances in the second group in general act as excitants in higher organisms. A number of other very interesting details of fact and conclusion are presented in the paper, but lack of space forbids mention of them here.

R. P.



## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt,  
Harvard University Medical School, Boston, Mass.

**Buxton, B. H.** Enzymes in Tumors. *Journal of Medical Research*, 9: 356-371, 1903. It has been shown within recent years that in addition to the extracellular

enzymes which are secreted into the digestive tract, there are intracellular enzymes in the animal body, in organs not concerned in the formation of the digestive juices. Buxton did not use filtered extracts owing to the difficulty of obtaining sufficient amounts of cancerous tissue. Instead, he tested for the presence of enzymes by means of diffusion in agar plates. This method is simple, and he thinks it will prove trustworthy.

Each tumor was chopped into fine bits and ground up with pumice stone in a mortar. A soft paste was made by the addition of fifty per cent. glycerin in water. The pulp divided into equal parts was placed in two sterile bottles labelled A and B. The bottle B was boiled for 20 minutes and more glycerin added to restore the mixture to its original consistence. To each bottle a little chloroform was added. At the end of two or three weeks the pulp became sterile.

A two per cent. agar was prepared. When needed it was melted and a little thymol added. After it had cooled down to 50°C. or 60°C. the substance required for the reaction was added. About 15 c. c. of agar were poured into each Petri dish. A small spoonful of the pulp from each of the bottles was placed on the agar and arranged in the form of a circle 20 mm. in diameter. One semi-circle was composed of pulp from A, the other half of pulp from B. The Petri dish was kept in an air tight incubator at 40°C. and exposed to vapor of chloroform water.

*Proteolytic enzymes.* (a) Egg albumen. Suspension of 5 per cent. of white of egg in agar. If a proteolytic action occurs the slightly opalescent agar becomes clear. None of the tumors acted upon the egg albumen. (b) Milk. Agar, 90 per cent.; sterilized milk, 10 per cent. Varying results were obtained with different tumors. Some produced coagulation (rennet) with subsequent clearing. (c) Gelatine. Ten per cent. gelatine with a little thymol. Some tumors caused liquefaction of the gelatine.

*Amylase.* Two kinds of agar plates were used. One contained 0.5 per cent. of soluble starch, the other 0.25 per cent. of glycogen. At the end of 48 hours a 3.5 per cent. solution of HCl is poured over the plates and then drained off, and diluted Lugol solution poured on. Except where sugar has formed the starch is colored blue and the glycogen brown. All the tumors caused some fermentation.

*Lipase.* One per cent. butyric acid in litmus agar. The medium should be just alkaline enough to color the litmus blue. If lipase is present in the substance tested fatty acids will be formed and the litmus changed thereby to red. This reaction occurred with all the tumors, but normal organs probably have the same action.

*Oxidases.* An emulsion is made by adding to a little distilled water in a porcelain dish some saturated alcoholic solution of gum guaiac. The dish is tilted and some tumor pulp placed at the margin of the fluid. If oxidation occurs a blue color appears. With none of the tumors did this reaction take place.

*Peroxidases.* On adding a little  $\text{H}_2\text{O}_2$  to the guaiac fluid a blue color is formed if peroxidases are present. All the tumors responded to this test, but so do normal tissues.

J. H. P.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn,  
Wesleyan University, Middletown, Conn.

**Jensen.** Studien über das Ranzigwerden der Butter. Jahr. d. Sch., 1901.

Many bacteriologists in the last 15 years have studied the problem of the rancidity of butter, and a variety of conclusions have been reached. Whether the phenomenon is purely chemical, is due to an enzyme or to the growth of bacteria has never been satisfactorily settled. Jensen has undertaken an investigation of the subject in the light of the facts hitherto obtained, and has carried on a series of exceptionally careful experiments. His evidence is obtained from a chemical study of the product and not, as has been the case with most previous experiments, from physical properties of the butter alone. The chief results which he reaches are as follows:

1. The air plays a part in the process of rancidity, but is not the cause of the change in question. It acts rather as a stimulant for bacteria growth.

2. The rancidity of butter is due to the action of micro-organisms.

3. The chief causes of rancidity are the well known *Oidium lactis*, *B. fluorescens laquifaciens*, and a form found by him universally present in butter, *Cladosporium butyri*.

4. These organisms grow in the presence of oxygen and not in its absence. To protect butter from rancidity, therefore, it is desirable to keep it in large masses, under which conditions the inner parts of the butter will remain without rancidity though the surface may change.

5. The presence of salt checks the growth of the bacteria and thus delays the rancidity.

H. W. C.

**Gorini.** Ueber die säure-labbildenden Bakterien der Milch. Cent. f. Bac. ii, viii, p. 137, 1902.

This author has previously shown that *B. prodigiosus* produces not only a certain amount of acid but an enzyme with a power of curdling milk similar to rennet. His method of demonstrating this was to cultivate the bacteria in question in milk and then to filter the culture through porcelain; the filtration was found to possess the power of curdling milk in a few minutes, indicating the presence of an enzyme. It occurred to the author to determine if possible whether among the normal bacteria in milk there might be some that had this same power. An examination of a number of samples of milk has shown him the presence of organisms with these two properties. These are cocci of apparently four or five different types. He is therefore inclined to recognize three classes of milk bacteria; one producing acid, a second a rennet like enzyme, and a third class producing both acid and rennet. The latter he believes may have an important bearing upon the problem of the ripening of cheese.

H. W. C.

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Journal of

# Applied Microscopy and Laboratory Methods

Edited by L. B. ELLIOTT.

**SEPARATES.**

One hundred separates of each original paper accepted are furnished the author, gratis. Separates are bound in special cover with title. A greater number can be had at cost of printing the extra copies desired.

WE are pleased to be able to announce, beginning with our next issue, a new Department in the JOURNAL, to be called "General Laboratory Methods." This Department will be conducted by Dr. Raymond Pearl, of the University of Michigan, Ann Arbor, Mich., and will consist of notes from current literature, dealing with laboratory technique in a more general way than has been possible in other Departments which are confined more exclusively to microscopical matters. The addition of this Department is in line with the broader policy of the JOURNAL, and will give our readers each month a very considerable amount of material of a kind which we have never before had. We would suggest in this connection that separates of all papers on general zoological subjects, in which there is a method described, be sent to Dr. Pearl for review.

WE have recently completed arrangements with Mr. B. J. Howard, U. S. Department of Agriculture, Washington, D. C., whereby we will be able to present to our readers in the near future, a series of papers on the use of the microscope in the detection of food adulterations. The series will be, to a large extent, a compilation of the methods which have been published in foreign languages during the past few years, and will serve when completed as a manual for this line of work. We trust that this will be the first of a series of manuals which we are arranging for, showing the application of the microscope in the industries. The industrial application of the microscope is being rapidly developed, and we shall use every effort to keep our readers posted in the various lines in which its usefulness has been fully demonstrated.

WE offer our readers in this issue a most valuable series of papers, recently presented to the Michigan Academy of Science, and published by the Academy for distribution to teachers in the secondary schools in the state of Michigan. Our object in so doing is to give our readers not only the information which is contained in these papers, but also an insight into the methods which are being employed by our science organizations throughout the country not only to further the interests of science, but to make available to those who need them most the practical results of scientific work and investigation. In addition to the publication of this series of papers, the Michigan Academy of Science has established a natural history bureau for the encouragement of the collection identification and exchange of natural history material throughout the State. Certain members of the Academy, and others interested in its work, have agreed to identify gratuitously material sent them from the schools.

The teachers are urged to start their pupils collecting along some one or more of the lines for which provision to identify the material has been made, the object being to get the pupils started before the close of the school year, so that they will continue the work during the summer, and have, when school begins again in the fall, a large stock of material ready for study, identification, classification and exhibition in the school museum. The Committee, however, does not recommend the collecting of bird skins, bird eggs or birds' nests on account of the destruction of the birds, which would thereby be encouraged. This is a beginning from which, doubtless, many other lines of work will develop, and we shall be glad to keep our readers in touch with the results accomplished by the Academy along these lines. In nearly every state there is a body of enthusiastic workers meeting together annually, presenting the results of their labors, results which would be invaluable not only to those who attend the meetings and hear the papers read, and to those who read the papers through the medium of the printed Proceedings, but to the entire body of science teachers of that state. Would it not be of even greater importance to see that these results are placed in the hands of science teachers in such a form as to be available by them for every day work, than to place them in the hands of those who now ordinarily receive them?

The JOURNAL will be pleased to hear from other states as to what is being done in this line, and to offer its services, as far as practical, for the dissemination of this information.

## NEWS AND NOTES.

**A NEW TEST FOR ALBUMIN.**—This new and simple test is based upon the following facts: (1) Albumin is coagulated by carbolic acid; (2) Equal volumes of non-albuminous urine and a mixture, composed of equal parts of carbolic acid and glycerin, form an emulsion which clears up entirely upon agitation, leaving a perfectly transparent and highly refractive liquid; (3) Equal volumes of albuminous urine and the above mentioned carbol-glycerin solution, when mixed together, produce a white turbidity, which remains, in spite of agitation, and does not precipitate on standing, nor redissolve.

The test is very sensitive, distinctly showing the presence of 0.1 per cent. of albumin in the urine, the degree of turbidity being proportionate to the percentage of albumin contained in the urine.

The method of applying the test is very simple, and requires as little or less time than the other reliable tests for albumin, and, as the carbol-glycerin solution is a non-irritating mixture, it is much to be preferred to the disagreeable nitric acid.

The solution consists of carbolic acid and glycerin, equal parts. Glycerin is added to prevent the formation of a permanent emulsion between the carbolic acid and the watery element of the urine, and is added in excess so that no possible error can occur from this cause.

*Test.*—2 c. c. of carbol-glycerin solution are poured into a small test tube, and 2 c. c. of the filtered urine are added. Mix thoroughly with a glass rod, or agitate. If a clear, transparent liquid results, there is no albumin present; but if the slightest turbidity is noticeable, the urine is albuminous.—*Med. Rec.*



# Journal of Applied Microscopy and Laboratory Methods

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# Journal of Applied Microscopy and Laboratory Methods

VOLUME VI.

JULY, 1903.

NUMBER 7.

## On the Use of Compression in the Study of Small Organisms.

This article is intended to give an account of methods which have proved useful in the study of small soft animals such as Hydra and certain flukes, and is offered to the readers of this journal, not as an original contribution to anatomical technique, but to call these methods to the attention of any who may not know about them or may not have been making as much use of them as they deserve. It may be supposed that the reader has a working acquaintance with the methods employed in studying whole specimens, either alive or after technical treatment.

To take the case of living Hydra first: a large gathering of Hydras is made and the well expanded specimens are looked over carefully with the aid of a large reading glass, so that those showing the organization most completely, as to buds, gonads, etc., as well as size, may be used. The specimen is removed with a pipette to the center of the stage of a compressor. I have found the instrument sold by Bausch & Lomb, the one designated as "life box with spiral slot," the most convenient instrument for this purpose; the cover is capable of being screwed down, its motion governable by the slot, and different degrees of compression obtained as desired. By observing the animal under the microscope during this action, the amount of compression needed to demonstrate the organs, and the degree which the organism can sustain without damage is regulated.

Frequently long continued observation is needed before many desired details of the structure, especially in a complex organism, come out into view. I have noticed this repeatedly in studying the trematodes. Thus, in studying *Cotylaspis* a form common in Anodonta, it was only after several hours' compression and continuous observation that I found it possible to recognize the details in the structure of the excretory system, details which cannot be ascertained at all in any other way, as they do not show in preserved specimens however they may be treated. Looss, the great student of the trematodes, recommends enclosing trematodes under a cover-glass in water in a ring of an oil, hardening at ordinary temperatures, to prevent the evaporation of the water, so that the worm can be kept for several days, and he says that he made most of the observations

recorded in his remarkable work on the distomes of fishes and frogs upon living specimens in this way (Looss, *Die Dist. Fisch. u. Frosch. Bibl. Zoöl.* xvi. p. 3, 1894).

It is not, however, necessary to have an expensive life-box or compressor to employ this method successfully. In many instances a cover-glass of the ordinary sort can be directly applied over the animal located beneath its center, and the capillary attraction between the slide and cover relied on for the compression. The degree of compression can be regulated by withdrawing water from beneath the cover-glass with a piece of bibulous paper at the edge, and removing it at the proper moment, or by adding water at the edge from a pipette. This simple device serves very well for brief examinations, but on account of the constant evaporation at the edge of the cover, it is not satisfactory where a specimen is to be kept under observation during several hours, in which case the life-box should be employed.

Living specimens of *Hydra* compressed in either of these ways demonstrate many points in the anatomy and histology very clearly indeed. Thus, the ordinary ectoderm cells and the nematocysts, the endoderm cells in different parts of the body and their differences in each locality are visible.

In many of the smaller trematodes in the living state this method is very successful indeed. It is, in fact, the best general method for studying these forms. I have in mind at present its employment in the study of *Cotylaspis*, a small fluke related to *Aspidogaster* and a frequent parasite of the fresh-water mussel, in which it lives on the cloacal surface of the kidney. It is a small animal, measuring about 2 mm. in length. By studying living specimens under compression, all the organs and their connections can be by degrees demonstrated. The details of histology are some of them shown in this way, too, and this is of advantage for the sake of the corroboration it furnishes to the information derived in the study of sections which have been subjected to the operation of a great number of different reagents. The intestinal epithelium, for example, is clearly seen and flame cells and the linings of the excretory capillary and their cilia are recognizable. The larger flukes, even such a large one as *Distomum* (*Fasciola*) *hepaticum*, while too large to permit as good results as the smaller forms, will, when studied by this method, show a considerable part of the organization and greatly help one in the interpretation of sections used to supplement the method.

The process of compression can be applied to objects already preserved, though not so effectively as to fresh ones. Thus, some time since, I wished to put up a set of whole specimens of the common liver fluke for use in a zoölogy class, from material which had been furnished me in alcohol. The worms had died in any position they happened to assume. To correct this, I first soaked them for a day in warm (30° C.) weak (30 per cent.) alcohol, till they were flaccid and soft, then shaped them and placed them between two glass slides and compressed them into a flat thin condition, as much so as could be done without injury to the structure. Specimens thus compressed became quite thin, and after subsequent handling gave excellent results. The later treatment was as follows: First they were placed in strong alcohol while still under compres-



sion and so hardened again. They were then soaked in 50 per cent. alcohol to prepare them for borax carmine, and then stained for one day in borax carmine. The point here is to overstain them very strongly and then to draw the color as desired with acidulated alcohol. For the decolorizing it is commonly recommended to use 2 per cent. hydrochloric acid in 50 per cent. alcohol. But I find it preferable in decolorizing whole objects to use a much stronger solution of the acid, which works more rapidly and so decolorizes the surface parts quickly and before the inner organs have been much affected, leaving the organs within more conspicuous than they would be if the whole organism were decolorized slowly. The action of the acid must be checked constantly by submitting the specimen to microscopic examination; the best results are only obtainable by carrying on the whole decolorization on the stage of the microscope and under one's eye all the time. Experience will soon teach one when the coloration is most satisfactory. It is also to be remembered that specimens to be mounted in balsam will need to be more deeply colored to compensate for the superior translucency of the specimen after clearing. After this the usual technique is followed, dehydrating, replacing alcohol with oil, and mounting in balsam.

Where it is possible to get living material which it is desired to study after treatment with reagents, it is very much better to compress it while still alive and then kill under compression. I have found that I have had the most satisfactory results in studying *Cotylaspis* and other flukes by using saturated aqueous corrosive sublimate solution as a killing fluid. I have proceeded in the following way: First the fluke is located on the slide and nearly all the water surrounding it removed with a piece of bibulous paper; a cover-glass with a drop of the sublimate solution in the center is inverted over the worm and suddenly let down. The amount of the solution determines the amount of compression, but as the animal does not harden instantly the compression can be somewhat regulated, after the cover has been lowered, by additions or withdrawal at the edge. This should be managed while the specimen is being viewed under a low power to determine just the point of compression desired. Specimens thus killed under compression are afterward hardened in serial alcohols and preserved in 70 per cent. alcohol till needed for study, when they are stained, dehydrated, and cleared in the usual way. This method, successfully applied, will give one wonderfully clear and distinct views of the internal structure of a trematode. It can be applied to the study of certain organs of other forms or to whole animals.

HENRY LESLIE OSBORN.

Biological Laboratory, Hamline University.

## Field Work in Zoölogy.

How we can best familiarize our children with plants and animals in their natural surroundings, is one of the problems still unsolved by the instructors of biology in great cities. The large number of students in each class, the distance between the school and the places where this work can be carried on, and the nervous strain upon the leader, are some of the reasons which militate against the success of this work. In spite of these objections, however, all will agree with me that field work is most valuable in all natural science teaching. It brings the children in touch with nature and her many secrets. They learn to observe more closely and it may lead them to place a higher value on the life of the lower animals, many of which are of economic importance to man. Interest is always a great educational factor and one of the best ways of arousing it is to take children out to study living things.

While we are conscious that we have not yet attained the best method for doing field work, some of our excursions may be of interest to those who have a similar environment.

The Morris High School is fortunate in being located in the upper part of the city, where it takes but five or ten minutes to reach a field. Our course has been outlined in this journal for May. We have five periods per week of forty-six minutes each. Our time schedule is so arranged that each class has the last period for biology at least once during the week. I advocate one double period. If it is not seasonable for outdoor work it could be used for laboratory work. Biology is taught in the first year, so the children are rather immature. Experience has taught us that they gain more, when working outside of school, by having some topic assigned with definite questions which they are required to answer on paper.

During our insect work each class was taken for one period to a field in the immediate neighborhood. Here they could study the living specimens. The following topics were suggested for observation: the home, habits, methods of locomotion, food-getting, and adaptations for protection. Specimens were obtained by the pupils for class-room work. In their efforts to secure grasshoppers and butterflies they had the method of locomotion well illustrated. The next day these topics were discussed in class and their notes written up more carefully. Dead specimens were now used for the study of the external morphology. Then the functions of insects were taken up, special attention being paid to the adaptations of the bee and butterfly for the cross-pollination of flowers.

A small class of boys went out to collect water insects. Their joy was great in being permitted to drag the pool of water with their nets. The fresh water aquarium that we stocked on our return has been of interest to them all winter.

In studying the earthworm the pupils were requested to study the home and habits of the animal. Living specimens were brought in for class work. A number of them were put in a large glass jar containing moist earth, some ferns and leaves, so the pupils could observe their habits. Since then two large black snakes have been added to the jar.

The frog was studied in a similar way, including two other topics for individual observation, its food and enemies. The economic importance of the earthworm and frog was brought out during these investigations. This work was done in the vicinity of the students' homes without the aid of the teacher. The results of their observations were written up and brought in for class discussion. The interest aroused was very great, even extending to the parents. Their indoor work is carried on with more zest when they have personally studied the activities of the animal in its natural environment.

A class of thirty was recently taken to the Aquarium, where they could see other specimens of the same groups that they had been studying. The Arthropoda, Amphibia, Cœlenterata, Echinodermata, Porifera, and Fishes appealed to them, because they knew something of their habits and structure.

Late in the term's work each class is taken to the Zoölogical Park in the Bronx, to study the animals there. Each student has a printed paper containing a few definite questions, under the following headings: Habitat, locomotion, food-getting, means of protection, sense-organs. Individual observations are made and notes are taken, which are discussed in class next day. We do not visit all the animals, but work on a few, such as the buffalo, wolf, bear, reptile, and bird, then observe the others. A few carefully examined will produce better results than if we attempted to do many. Frequently the animals are fed for the benefit of our classes and the attendants are always very kind in showing the students the most interesting specimens. The pupils work with much pleasure.

When we have completed the study of the human skeleton, each class is taken to the Museum of Natural History to make a comparative study of the mammalian skeleton. They use Peabody's Laboratory Exercises in Anatomy and Physiology, being required to answer in writing the twenty-nine questions under the following headings: spinal column, ribs and sternum, anterior appendages, posterior appendages, teeth.

Each student selects three animals upon which to work. Several may work on the same one, which prompts discussion, for all are not equally keen in seeing the number of vertebræ in the cervical region, or whether the animal has a clavicle, etc.

We are fortunate in having a Zoölogical Garden, Aquarium, and Museum in our city, but much valuable work could be done by schools that are located in districts near the country. A teacher could take her class to some place where cows or sheep are kept. By carefully observing one animal they may become interested and examine others of a different kind.

Encourage your students to bring in specimens of all kinds, it not only teaches them, but arouses the interest of other children.

We have in our classroom a salt water aquarium containing sea anemones, shrimps, and snails. When working on the crayfish the shrimps were useful for comparison. There are several fresh water aquaria, one containing water insects and tadpoles in the first stage of development, another contains tadpoles in more advanced stages, others contain newts, turtles, an alligator, and crayfish. A large wooden box was fitted up for the frogs. The pupils have been encouraged to

experiment with the various specimens to see what would live together. The classes seem to have a live interest in their work, and I feel that during the coming summer they may be more observant of the animal life which surrounds them.

We hope this year to do some work on the economic importance of birds, trusting that it may be one step toward their preservation in the future. If we can inculcate the right spirit into our boys and girls, it ought to be a great factor in their attitude toward the birds.

This outside work means great fatigue upon the part of the teacher, but all who have tried it must consider it one of the most valuable parts of their work. It frequently fosters a liking for animals which was lacking before. The child who feels disinclined to take part in this work often becomes our greatest enthusiast. The students who have considered the museum an uninteresting place, have been heard to remark, "Why, I did not know it was like this," or "This is not work."

The teacher and pupil are brought into a more sympathetic relation, which simply emphasizes the fact, "That one touch of nature makes the whole world kin."

KATE B. HIXON.

Morris High School.

## Flies as Carriers of Bacteria.

*School Science* for April publishes an interesting laboratory study in elementary bacteriology by Eva May Shoemaker and Alvin Waggoner, two juniors, in the Eastern Illinois State Normal School. These two students, working in a secondary school laboratory, tried to determine experimentally "the part flies may play in the transmission of bacteria."

To do this they constructed a piece of apparatus, consisting of a box divided into two compartments (Fig. 1). In the first compartment they exposed food material infected with an easily recognizable species of bacteria,—harmless bacteria, of course, being used,—and in the second compartment they placed an open Petri dish, containing sterile nutrient agar. Flies were placed in the first compartment, and, as soon as a number of them had been seen to walk upon, or eat of the infected material, they were allowed to pass through a small door into the second compartment, where they had a chance to come into contact with the agar in the Petri dish. Bacteria, deposited on the surface of nutrient agar, multiply there and form characteristic colonies.

"In the preliminary experiment, performed July 16th, a yellow bacterium was used. Molasses mixed with a growth of this bacterium was spread on a plate in the first compartment, and a dozen flies put into the apparatus. Half an hour later, the door between the two compartments was opened, and the flies allowed to pass through. As soon as six of them had been seen to come in contact with the agar in the Petri dish, the dish was covered and put away to develop. A few days later, there had grown on the agar over one hundred yellow colonies of bacteria."



They repeated this experiment with red and with violet bacteria, and obtained characteristic red and violet colonies.

To prove that the germs from which these colonies grew came from the infected material in the first compartment, and not from accidental sources, control experiments were made with other groups of flies, but with no infected material in the first compartment. In no case, however, did the Petri dishes used in these control experiments develop yellow, red, or violet colonies.

To prove that the flies were the only means of transmitting these bacteria from the first compartment to the second, experiments were made with infected material in the first compartment, but with no flies in the apparatus. The Petri

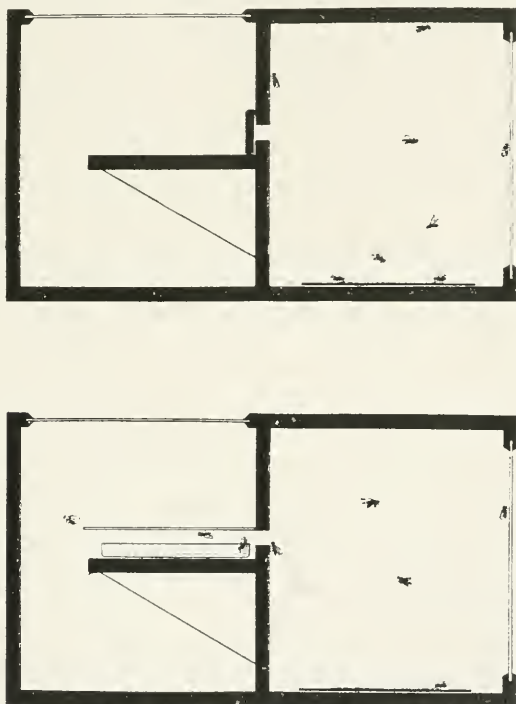


FIG. 1.

dishes from these experiments, also, developed no colonies. "The germs that grew into colonies in the first experiments, therefore, (i) came from the infected material in the first compartment, and (ii) were carried to the Petri dishes by flies." From these results, they drew the more general conclusion, that, "*flies are capable of carrying bacteria from one place to another, if they have a chance to come in contact with material containing these organisms.*"

They next undertook to determine "whether flies in nature actually do carry bacteria with them." "To determine this, test tubes of sterile agar were melted and then cooled to 40° C., a temperature a little above that of the human body. At this temperature the agar still remains liquid, but is not hot enough to kill bacteria. Flies were caught with sterile forceps and washed in this melted agar.

The agar was then poured into sterile Petri dishes, where it solidified. . . . It is reasonable to suppose that if bacteria were present on the flies, some of them, at least, would be washed off by this process into the agar, where they would multiply and form colonies."

The dishes obtained from these experiments varied greatly, but all of them developed colonies, the number ranging from fifty to over two thousand. "*Flies in nature*," they conclude, therefore, "*probably always do carry bacteria with them*."

"From the standpoint of public hygiene," they continue, "these conclusions are very significant; for, admitting that flies can carry harmless bacteria, there seems to be no reason why they cannot carry disease-producing bacteria as well. In fact, it has been a general belief among scientists for a number of years that they are active agents in the transmission of many diseases. Recent studies by Dr. L. O. Howard on the breeding habits of these insects furnish good evidence that they occasionally play an important role in the transmission of typhoid fever. It is probable that they can, and do, carry the germs of any disease that offers them an opportunity to come in contact with infected material."

"Their work is of value," says *School Science*, "not only on account of its scientific interest, but, also, because it points the way to a new field of effort open to secondary students,—a field whose development would have an important influence on educational methods, as well as on public health problems."

Johns Hopkins University.

W. H. MANWARING.

## Preparations for Dissecting Pans.

In view of the fact that such preparations for dissecting pans, as are on the market at present, are either expensive or not very serviceable, or both, it was thought worth while to attempt to work up some mixture that would be more serviceable and less expensive.

An ideal preparation for dissecting pans, it seems to me, should be of such a nature that it will hold the pins. It should be soft enough so as not to crack when bent, adhesive enough to stick to the pans, black enough to form a sharp contrast with delicate tissues, and insoluble in solutions (water, 70 per cent. alcohol, and formal) commonly used in dissection.

After considerable experimenting, two preparations were obtained, neither of which prove to be ideal. The formulæ of these two preparations (A and B) are as follows :

### PREPARATION A.

25 grams of Linseed oil,  
50 " " Coal tar,  
200 " " Brown resin,  
50 " " Hard paraffin.

### PREPARATION B.

50 grams of Coal tar,  
100 " " Brown resin,  
250 " " Hard paraffin.

The ingredients of preparation (A) mix most readily if all but the paraffin is first melted and thoroughly mixed, and then the paraffin added and melted and the whole thoroughly stirred. This preparation may be made harder by con-

tinuous heating or by decreasing the amount of linseed oil, and softer by increasing the proportion of linseed oil.

As in preparation (A) so in preparation (B), a more thorough mixture is obtained if the coal tar and resin are first well mixed and then the paraffin added, than if all the ingredients are at once added together. This preparation requires more heating and stirring than preparation (A) in order to cause the ingredients to mix; and at best there will be only a partial mixture. Coal tar and resin being heavier than paraffin tend to collect at the bottom, so that in this preparation it was found best to weigh out the desired amount of substances used, in proper proportion for each pan separately, and melt and mix them in each pan.

As above stated, neither of these preparations is ideal; both lack some essential characteristics. Preparation (A), while insoluble in water and formal, is slightly soluble in 60 per cent. alcohol, and quite readily soluble in strong solutions, and consequently is not satisfactory for dissection in solutions of alcohol stronger than about 50 per cent.

Preparation (B) is practically insoluble in 96 per cent. alcohol as well as in formal and water, and is consequently satisfactory for dissection in these solutions; but this preparation does not adhere to the pans as tenaciously as preparation (A), and it might also be improved by being made slightly darker in color.

COST.—Coal tar and resin are usually sold by dealers in plumbing material, the former at 25c. per gallon, and the latter, at 5c. per pound. Linseed oil is handled by dealers in paint. It is retailed at from 40c. to 80c. per gallon. Paraffin may be obtained from druggists or dealers in laboratory supplies, at about 20c. per pound.

According to the above prices the approximate cost of the preparations would be as follows:

#### PREPARATION (A).

1 lb. Linseed oil,	-	-	-	-	-	\$ .08
2 lbs. Coal tar,	-	-	-	-	-	.08
8 " Resin,	-	-	-	-	-	.40
2 " Paraffin,	-	-	-	-	-	.40
						<hr/>
Total, 13 lbs.	-	-	-	-	-	\$ .96
Cost per pound, 7 $\frac{5}{7}$ c.						

#### PREPARATION (B).

2 lbs. Coal tar,	-	-	-	-	-	\$ .08
4 " Resin,	-	-	-	-	-	.20
10 " Paraffin,	-	-	-	-	-	2.00
						<hr/>
Total, 16 lbs.	-	-	-	-	-	\$2.28
Cost per pound, 14 $\frac{1}{4}$ c.						

The amount of either of the preparations required to cover the bottom of a dissecting pan 5 x 8 inches, about  $\frac{5}{8}$  inch deep, weighs approximately one-half pound, making the cost of preparation (A) per pan about 4 c., and of preparation (B) about 7 c.

S. O. MAST.

## Methods of Cultivating *Amoeba* and Other Protozoa for Class Use.

One of the periodical problems with which every teacher of zoölogy is met is that of having on hand at the proper time an abundant supply of *Amœba* for class use. Certain methods by which various teachers have solved this and similar problems have been published in this journal, and have been very welcome. A method differing from any of those yet given has been used with much success by the writer for furnishing classes of eighty or more students at any desired time with a plentiful supply of *Amœba*, as well as of almost any other of the common Protozoa. The method is as follows: Two weeks or so before the specimens are desired, bring from a river or pond a large quantity of some of the aquatic plants which grow everywhere. If possible, obtain *Ceratophyllum*, though *Elodea* and various other plants will do (*Potamogetons* are not satisfactory). Put this material into dishes, not too deep (circular "crystalizing dishes" or "bacteria dishes," three inches deep and eight or nine inches in diameter, are convenient). The dishes are to be crowded full of the plant material, which is then covered with water and left to decay. The dishes may be covered by a glass plate. To be certain of a large supply of *Amœbas*, a dozen or twenty such cultures should be set in operation. After a certain stage in decay has been reached, the layers of the plant at the surface of the water become covered with a brown slime or scum. In this brown slime will be found, in some of the dishes, many *Amœbas*. Not all the dishes contain the animals, but if a dozen or more dishes have been provided, after two weeks or thereabouts, some of the vessels will be found to contain unlimited numbers of *Amœbas*. For the last three years the writer has supplied a class of eighty to a hundred in this way, and has never known it to fail. Dozens of *Amœbas* will sometimes be found in the field of view when a bit of this brown slime is examined. It is well to remove a bit of the plant and scrape the slime from this; in the slime on the surface of the water fewer *Amœbas* will be found. The points to which special attention should be paid, if *certain* results are to be obtained, are: (1) be certain to provide a sufficient number of cultures; (2) begin at least two weeks beforehand; (3) it is well to add a few dishes of new material every four or five days after the first was obtained, so that there will always be cultures in the proper stage. In a given culture frequently the *Amœbas* last in quantity only two or three days.

The above method is, of course, rather a rough and ready one than an elegant one, but this is its recommendation. It relieves one, at the cost merely of a little industry, of all anxiety as to whether there will or will not be material on hand when it is required.

*Arcella*, *Diffugia*, *Chilomonas*, *Chlamydomonas*, *Euglena*, *Stentor*, *Paramecium*, and many other rhizopods, flagellates and ciliates may be obtained in quantity from the same cultures, so that the latter form a general source of supply, even for very large classes, for most or all of the protozoan material needed in any ordinary laboratory course.

H. S. JENNINGS.

University of Michigan.



## A Simple Improvement of the Pillsbury Slide Box.

After several years experience with many kinds of slide trays and cabinets in the Zoölogical department of the University of Pennsylvania, we have returned to the use of Pillsbury boxes for all slides of ordinary size. Our reasons for this are that slides are rarely if ever broken in these boxes, whereas many have been broken by being shaken or jarred out of position while in the trays; the boxes are more compact than trays and much less expensive; they form convenient units which can be catalogued and placed on shelves like books to be receipted for when withdrawn, and, last but not least, the boxes may lie in any position or be transported to any distance without injury to the slides.

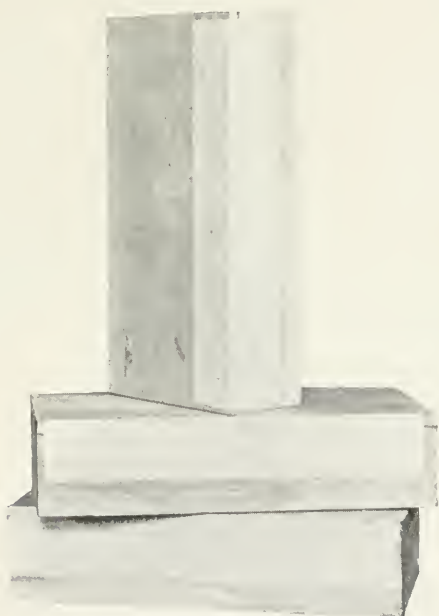


FIG. 1.

In our experience the most serious objection to the ordinary Pillsbury box is that the lids come to fit loosely after a while and when the box is standing on end drop off on the slightest provocation; and to make matters worse lids become interchanged so that they fit badly or not at all, thus allowing dust to enter the box; furthermore, if the box should be overturned all the slides are liable to fall out.

To obviate these difficulties some Pillsbury boxes are made with brass hinges and latches, but their cost is thereby considerably increased and such hinges and latches could not well be applied to boxes already in one's collection. In casting about for some inexpensive device by which the lid could be hinged to each box in our collection, I hit upon the simple expedient here described. This has been

in use in our laboratory for the past two years and is so satisfactory, so easily applied, so inexpensive, and so durable, that it can scarcely fail to be generally used wherever it has once been tried. This device consists simply in sticking a strip of gummed linen tape along one edge of the box and lid so that it will serve as a hinge. The linen strip should be about two inches wide and a little shorter than the box is long, and should be applied, while the lid is in position, along one edge of the box so that about one-half of the strip is attached to the lid and the other half to the box. It should be applied while quite wet, pressed down smoothly and allowed to dry before the lid is opened. If the lid does not fit tightly some weight should be placed on it to prevent its warping open while the

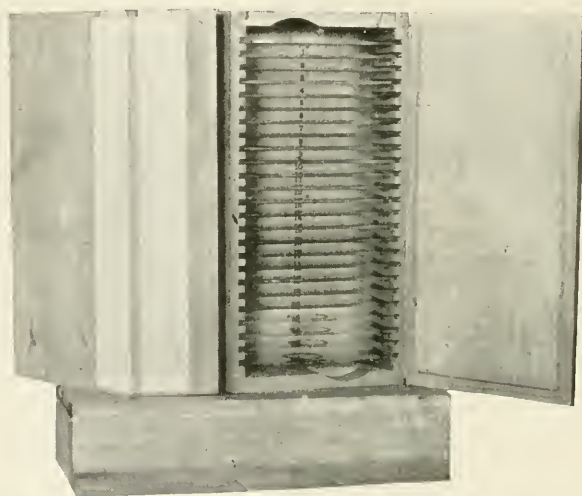


FIG. 2.

linen dries. Suitable gummed linen tape can be obtained in rolls varying in length from 20 to 100 yards, and at a trifling cost.

Such a hinge is as strong as a pair of brass ones (for the past two years, during its use on a large number of boxes in our laboratory, not a lid has been torn off or displaced) and it has this additional advantage, that it completely closes one of the cracks by which dust might enter the box.

An equally simple and effective latch was difficult to devise, and after having tried several kinds all were abandoned, as it was found that the lids fitted sufficiently well to remain closed without the aid of a latch.

The accompanying photographs (Figs. 1 and 2) show a few boxes with the "linen hinge" in position.

EDWIN G. CONKLIN.

University of Pennsylvania.

## Note on a Method of Preparing Sugar-Free Bouillon.

The method herein described has probably occurred to others, but not having seen it mentioned anywhere, I have taken this opportunity to present it, hoping that it may be of value to some of those who work a great deal with this medium.

The original method by which this bouillon was prepared, that of allowing the beef-infusion to undergo putrefaction, and at the same time fermentation, and the method which followed this, that of fermenting the already contaminated (ordinary putrefaction organisms) beef-broth with the *Bacillus coli communis*, are open to the objection that the putrefactive organisms (especially the *Proteus vulgaris*) present in the meat produce a very foetid odor, which makes the preparation and handling (the odor remains even after the medium has been boiled and sterilized) of this medium extremely disagreeable.

I found that, if the infusion was sterilized previous to the inoculation with the Colon bacillus, a perfectly odorless and sugar-free broth could be obtained. The method in detail is as follows: To a portion of the round of beef (lean), ground fine, add double its weight of cold water and bring slowly to a temperature of 50° C. over water bath. Maintain at this temperature for three hours, then strain broth through muslin. Put meat infusion thus obtained into sterile flasks and sterilize for three-quarters of an hour in live steam. After sterilization allow to stand in ice-box over night and then inoculate with a pure culture of the *Bacillus coli communis*. Cultivate from eighteen to twenty-four hours at 37.5° C. and then boil and filter to get rid of the extraneous meat particles. Now add  $\frac{1}{2}$  per cent. of NaCl and  $\frac{1}{4}$  per cent. of peptone and boil for three-quarters of an hour. Neutralize with NaOH, filter, put into sterile flasks and sterilize.

The medium thus obtained is odorless and completely sugar-free and may be used for the demonstration of the indol reaction or as a basis for the fermentation media (made by adding to it 1 per cent. of glucose, lactose or saccharose).

Bio-chemic Laboratory, Bureau Animal Industry.

F. E. MONTGOMERY.

## A Method for Staining Polar and Other Granules in Bacteria.

This stain was devised by J. F. Broderick, a member of the laboratory corps.

*Broderick's Method.*—Prepare a smear in the ordinary way on a slide. Add Loeffler's methylene blue, sufficient to cover the preparation  $\frac{1}{16}$  inch deep. Add pure undiluted hydrochloric acid, 1 to 3 drops, and tilt back and forth to mix with the methylene blue until an iridescent film forms on the surface of the methylene blue. Drain and add 3 drops of carbol fuchsin; tilt to mix; drain; add more fuchsin; tilt and mix until stain becomes a deep reddish-purple color, then drain and wash. The smear should be a light pink. The granules are deep red, blue red, or black, the rest of the cell faint red or pink.

This method stains the Neisser staining granules of *B. diphtheriae*, the granules, intracellular spores, and free spores of *B. megatherium*, and central and polar granules in *B. typhi*, *B. coli*, and many other species. The granules mentioned may often be seen in unstained hanging block preparations. By a special method, to be described later, individual bacteria may be focused upon, stained, decolorized, restained, etc., while under observation. By this method, it has been conclusively shown that the above described stain acts upon granules, etc., which are normal to the cell, and not upon artefacts due to the stainer's process.

HIBBERT WINSLOW HILL, Director.

Bacteriological Laboratory, Boston Board of Health.

## A Method for Moistening Court-plaster Strips Used in Anchoring Mounted Specimens.

The following simple contrivance (Fig. 1) for moistening the court-plaster strips used in anchoring mounted botanical specimens has been found to greatly expedite the work of anchoring.

Two small oblong pieces of wood are covered on one side with a layer of fine sponge, one-half inch or more in thickness. The sponge can be fastened to the boards with one ounce tacks. These sponge covered boards are fastened to a heavy block, in such a manner as to bring the sponge layers in close contact.

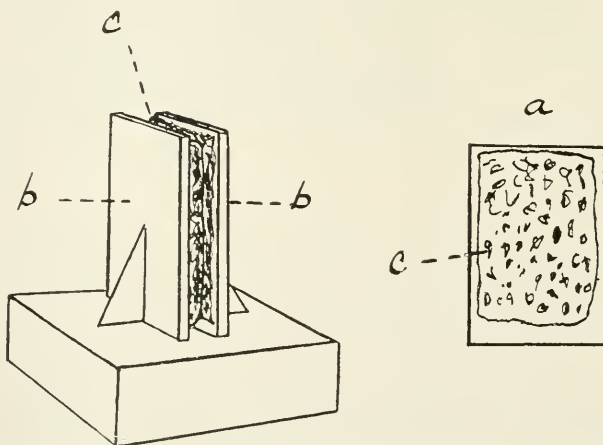


FIG. 1.—*a*. Front of sponge board showing sponge lining; *b*. Sponge covered boards in position; *c*. Sponge lining.

To use this apparatus, moisten the sponges thoroughly. With a pair of forceps grasp the courtplaster strip in the middle and draw down between the wet sponge lining, so the ends of the strip fold together, exposing the gummed surface to the sponges as it passes between them; it is thus sufficiently moistened to apply at once to the specimen. A soft cloth should be used to press the plaster strip both to the mounted sheet and close against the specimen.

Johnson and Johnson's surgeons' silk (isinglass plaster) moistens more quickly than any other brands which have been used.

Bureau of Plant Industry.

KARL F. KELLERMAN.



## Algae and Fungi for Class Work.

In the April number of this Journal Miss Mary A. Hickman reports the successful rearing of *Coleochæte* in the laboratory, and says that "it seems probable that *Coleochæte* may be obtained for laboratory work by this method." My experience shows that in this opinion she is correct. I have raised this alga in abundance without trouble; in fact, it is one of the most likely to occur in culture jars, or bottles filled with water plants. In one small bottle that stood on my table for several months during the past fall and winter, almost the whole side away from the light (which was rather strong) became covered with the circular discs of *Coleochæte*, which remained in healthy condition for a long time. Also in a large culture jar containing *Myriophyllum* and other plants a great amount of *coleochæte* appeared, here likewise on the less illuminated side, although the jar was a considerable distance from the window. It is generally difficult to remove this algæ from the glass without breaking it badly; and a good method to get it in nice condition for study would no doubt be to hang cover-glasses against the shady side of such culture dishes and allow the alga to grow attached to them. They could then be removed and studied without disturbance.

To get *Ulothrix* zoöspores take some of the alga from moist ground (not immersed), and place it in the bottom of a small jar of water. By the next morning zoöspores will probably appear in abundance. I have taken in nearly dry *Ulothrix* which, when treated in this way, produced zoöspores from almost every cell. According to my experience zoöspores of any alga are best obtained by introducing a sudden change in environment.

Another instance of zoöspore production being induced by changed conditions was found in experimenting with *Schizomeris*. A small bottle of this alga had been in the laboratory for a long time and had become somewhat foul. During this time no zoöspores were produced, but when the alga was at last removed to a bottle of fresh water, an immense number of zoöspores were formed in less than twenty-four hours.

It is remarkable how long some algæ can retain their vitality under most adverse conditions. As an illustration, a small vial about three inches high full of water containing a little *Nostoc* was tightly stoppered and packed away for about twenty months, and when again examined the *Nostoc* was still fresh and brightly colored, and showed no signs of bad treatment.

When a bottle of algæ is brought into the laboratory there is usually a fouling of the water due to bacteria, which is hard on the plants, but if the culture is let alone it soon clears up and the algæ renew their growth, and live and prosper for a long time without further attention. By this method I have had no trouble in raising young plants from the zoöspores of *Vaucheria*, *Ulothrix*, *Oedogonium*, *Aphanochæte*, *Coleochæte*, *Schizomeris*, and *Stigeoclonium*. *Tetraspora* and *Euglena* were also cultivated repeatedly.

Any of the cells of *Tetraspora* seem capable of swarming under proper conditions. In one case a number of nearly quiet cells in jelly were put in the sun

on a covered glass and watched with the microscope. Very soon a great number of the cells began active motion, escaped from the jelly (which was very thin) and hurried away to the dark side of the glass, where they crowded each other in great numbers. The swarm spores show every variation in size, and it is difficult to distinguish the smaller of them from gametes.

In studying the algæ, notes should be taken on their periodicity—time of appearance and disappearance in any given place. They show periodicity very strongly, and repay observation along this line (See Fritsch. *Algological Notes*, IV. Remarks on the Periodical Development of the Algæ in the Artificial Waters at Kew. *Annals of Botany*, Vol. 17, No. 45, 1903).

A serious drawback to the use of *Rhizopus* in class work is the difficulty, often impossibility, of obtaining its sexual reproduction, and a good substitute for it is needed. Such a substitute is to be found in *Sporodinia grandis*. There is no reason for failure to obtain the zygotes of this form if a little care is taken. Bring into the laboratory a number of moderately firm mushrooms, such as *Polyperus* or *Russula* (ones that do not deliquesce), and leave them exposed on the table, if the weather is not too dry. In a day or two *Sporodinia* is almost certain to appear on some of them as a yellowish fluffy growth, changing to dark brown as the zygotes are formed. When obtained, this fungus never fails to produce sexually. During the past fall and winter I have had no difficulty in getting conjugating stages, not only in *Sporodinia*, but also in *Rhizopus nigricans* and *Mucor heterogamus*.

W. C. COKER.

University of North Carolina.

## Keeping Earthworms Alive in Winter.

The period for studying the earthworm often comes, in its logical position in a zoölogical course, late in the fall or in the winter, when living specimens cannot be collected. It is therefore necessary, for some parts of the work, to keep a number of specimens alive. This may be done in boxes of earth, but frequently the results of this method are unsatisfactory, especially when a large number of the worms must be kept. If the earth is allowed to become for a short time too moist or too dry, the worms die, and in any case it is difficult to get at them when only one or two specimens are wanted.

A method of keeping them which obviates these difficulties was used by the writer with much success during the past winter. This is the very simple method which was recommended by Joest (*Archiv. f. Entw.-Mech.*, Bd. 5, 1897, p. 425) for cleaning out the intestine of the earthworm before sectioning. The worms are placed in a tight jar or other vessel, together with a quantity of damp clean cloth. I used bacteria dishes about nine inches in diameter by three inches in height, covered with a plate of glass. In each dish ten or a dozen worms were placed. Then strips of muslin were wet with clean water, the water pressed out as far as this could be done with the hand, and with these the worms were covered. The cloth must not be dripping wet, but only decidedly damp. The worms were kept in this way more than three months, and were

healthy and in good condition at the end of that time. The cloth needs changing or washing once in two weeks, or oftener, otherwise bacteria and fungi may get a foothold, destroying the worms. Possibly more specimens could have been kept in a single dish; with a larger vessel this would certainly be the case. The specimens are accessible at any time; the cover may be removed and one or more worms taken at any moment. The dishes were kept in the light, on the laboratory tables. This method would furnish excellent facilities for experimentation or continued observation of the worms; they may be fed on leaves, etc., if desired, and a given individual may be examined repeatedly whenever desired.

The method described above was devised by Joest for cleaning out the intestine of the worms preparatory to sectioning. For this purpose it seems to the writer much superior to any other. The worms cannot devour the cloth, but continue to void the waste matter, so in the course of a few days the intestine becomes entirely empty. The method is therefore much better than those in which the intestine becomes filled with paper pulp, bread, coffee grounds, or the like, which often cause serious difficulties in the sectioning. In order to insure the thorough cleaning of the intestine, the cloth should be changed every day, so that the worms may not again ingest the voided sand, dirt, etc. It is usually not difficult to tell by inspection when the worms have become entirely clean and empty.

H. S. JENNINGS.

University of Michigan.

### Soluble Glass as a Satisfactory Mounting Medium.

Having occasion to make frequent examinations of paper, and to prepare reference mounts of the fibers composing the sample, I have found soluble glass a mounting medium allowing of rapid and satisfactory work.

The paper under examination is softened by soaking in warm distilled water, and worked with the hand until reduced to a light pulp. A portion of this is then taken, and with the assistance of a low power lens and a pair of teasing needles, arranged on a slide.

The surplus water must now be removed, and the slide is, therefore, held over a flame until just sufficient liquid remains to wet the preparation evenly. The evaporating should be done carefully, so that bubbles will not be formed.

A drop of a thick soluble glass solution is then placed on the fibers, and a cover-glass laid on in the usual manner. The film of moisture, which is deposited by condensation on the cover-glass as it is lowered, is, to a great extent, the remedy for the numerous air bubbles, which, because of the uneven spreading of the soluble glass, would be formed were a dry cover-glass used, and which the rapid hardening of the medium would render impossible to remove.

The advantages of this method are: simplicity; durability of the preparations (provided they are kept from fumes of HCl) as indicated by about two years' use in the laboratory; infusibility of the medium, allowing of projection with any kind of illumination, and for as long a time as desired; greater dis-

tinctness, the fibers being rendered less transparent than when mounted in balsam; rapidity of hardening, the cover-glass being firmly fixed within fifteen minutes after attaching.

The great tendency to form air bubbles would seem to make the method objectionable, but where the object is not so much to produce ideally perfect mounts, as to produce them rapidly, this matter is not of much importance, especially as by practice and care but few bubbles of consequence will be found in the preparation.

CHARLES E. M. FISCHER.

Textile Laboratory, Western Electric Co.

## A Rapid Method for Hardening and Embedding Tissues.

Tissues can be readily hardened and embedded for cutting into sections in a hot solution of agar-agar containing formalin. The proportions of the mixture which have so far yielded the best results are 9 parts of a 5 per cent. aqueous solution of agar-agar to 1 part formalin. This mixture can be prepared beforehand and kept indefinitely in an air tight vessel. The agar-agar should be boiled for several hours, and after the addition of the formalin allowed to clear by sedimentation. The bits of tissue to be embedded are placed in a wide test tube or wide mouth vial containing the mixture previously melted. This is kept at 65 to 70°C. for an hour or longer, and the tissues are ready to be blocked. After attaching to blocks, they are placed in strong or absolute alcohol for an hour or two and can then be cut. It is important not to use dilute alcohol. The tissues are stuck to the blocks by means of the agar-agar itself, and adhere as soon as the agar becomes cold. No previous hardening of the tissues is at all necessary; fresh tissues can be placed at once into the hot agar-agar formalin mixture; in fact, fresh tissue is more desirable than that which has been previously hardened, though these can also be readily embedded by this method. But the main advantage of the method, aside from its convenience and simplicity, is the fact that the cells of the tissues are not at all contracted or shrunk, and the ordinary methods of hardening do have this effect more or less. With sections prepared from fresh tissues by this method the cell-protoplasm fills out the membrane fully, and the granules of the protoplasm, the nuclei and the cell contours are remarkably distinct. The whole process, hardening, embedding and cutting, does not occupy more than three or four hours, where the tissues are not larger than about 1 cm. in diameter.

Marion Sims College of Medicine.

B. MEADE BOLTON and D. L. HARRIS.



## The Technique of Biological Projection and Anesthesia of Animals.

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### XV. THE TECHNIQUE OF COLLECTING, MOUNTING, AND PROJECTING LIVE PLANTS AND ANIMALS.

This part of our subject presupposes a working knowledge of the essentials of projection microscopes and of the methods of anesthetizing animals, as described in the preceding articles of this series. We are now to consider in detail the kinds of apparatus needed for collecting, keeping alive, and mounting various types of small live organisms, the methods of using the apparatus so as to attain the best results with the expenditure of a minimum amount of time, and the difficulties to be overcome in manipulating the mounted specimens on the projection microscope. The work is not difficult and the results are both interesting and valuable. A large number of species of small aquatic animals, not usually studied in zoölogical courses but especially serviceable in this work, may be readily collected along with the species more commonly studied.

#### AQUATIC SPECIES.

These are found in greatest variety and abundance among submerged plants in slow streams, ponds, and lakes. The stock of live material for winter work should be collected in late summer or early fall, and is easily obtained with a simple outfit consisting of a strong fishing rod from eight to twelve feet long with a strong screw-hook of medium size securely fastened at its tip; a flat bot-tomed basket filled with six or more glass fruit cans, without covers, and several wide-mouthed bottles of different sizes; a pocket magnifier with lenses of from two to four inches focal length; a wide-mouthed bottle or slender beaker holding about half a pint, made of such clear glass that small animals may be easily seen through its sides, and fitted with a bail of strong cord; a pocket knife with a sharp blade of medium or large size; and a fine-mesh wire strainer about four inches in diameter, or a fine net of about the same size, which may be attached to the tip of the pole and easily removed when the hook is to be used. Dealers in house furnishings sell wire strainers of different sizes, and, in addition to the above, a small one about one and a half inches in diameter having a very fine mesh and a rigid handle is a most useful piece of apparatus for removing small and active animals from aquaria in the laboratory. Such an outfit is easily carried on a bicycle.

On arriving at a pond or sluggish stream find a place where submerged water plants, especially the common water-weed (*Elodea* or *Anacharis canadensis*), are abundant and fill the beaker with clear water. If the shore is soft and wet, the beaker may be hung by its bail on the screw hook and dipped into the water and set aside ready for use. Reach well down to the base of the stems of the water plants with the hook and firmly but gently pull them up, draw them to shore and immediately place all or a part of them in the beaker of water. It is usually

necessary to select some of the stems without roots and mud for examination in the beaker. On looking through the sides of the beaker with the magnifier one is frequently surprised at the variety of species and number of individuals it contains. Some species of active habits, e. g., *gammarus*, *daphnia*, leeches, and many insect larvæ are quickly seen, if present; others, e. g., *hydra* and *bryozoa*, contract when the plant is drawn from the water, but soon expand, if the plant has been transferred quickly to the beaker; other species, e. g., *spongilla* and the egg masses of snails, are recognized by their form and color. When satisfactory material is found, transfer it to the collecting jars with the water from the beaker. Many species are more abundant on wood that has been in the water for a long time. With the pole and hook draw such water-logged and floating pieces of wood to the shore, pull them out, let the water drain off and then examine for leeches, nymphs of dragon-fly, *gammarus*, snail's eggs, encrusting masses of *spongilla*, *bryozoa*, and larvæ of various species of insects either free or enclosed in cocoons. The blade of a pocket knife is used in transferring the free species to the collecting jars, and attached species are removed by cutting a thin shaving of the wood with the animals attached and placing it in the jar. Some species are too active to be captured except by rapidly sweeping the strainer or net through the water at different depths.

When making collections it is well to visit different localities in which the conditions are quite varied, swamps and ditches affording striking contrasts in fauna and flora with ponds and streams. In making collections do not crowd the jars full of plants. Place delicate, rare, or especially fine specimens in bottles or jars by themselves. Jars containing hydras should be free from such predatory species as the nymph of dragon-fly. Take the material to the laboratory and distribute it in battery jars, culture dishes, and other aquaria and expose them to a good light in a south window. After emptying the material into aquaria fill the collecting jars with clear water and examine for specimens of *hydra*, leech, and other species which attach themselves to the jar. If sheets of window glass are laid loosely over the aquaria, dust and dead flies are kept out and but very little water is needed to replace the loss by evaporation. By this simple and inexpensive method it is possible to keep a wide variety of species alive and in perfect condition for projection work at any time during the winter and early spring as well as during the months when collecting is possible. One of the species which is kept alive through the winter with the greatest difficulty is *hydra*, but success has attended the use of the above described method for three seasons. The most satisfactory results in growth and budding of the animals during the winter were obtained by placing strong specimens collected late in the fall in a tall and slender battery jar containing a few plants of *Chara fragilis* growing in a thin layer of pond ooze in the bottom of the jar. To make sure of an abundance of natural food for the hydras a large number of live water-fleas were taken from another aquarium by means of a small strainer and placed in the aquarium with the hydras. The jar was kept in a south window in a room which was allowed to cool off during the night and which became so cold on two or three occasions that ice an inch thick formed on the water of the jar. The jar was not moved, but was warmed up very slowly as the room temperature was

raised, and the hydras resumed their normal activities. The chara has been in fruit for months, and the jar has furnished an abundant growth in pure culture of a large desmid and a species of *Diffugia*.

Aquaria which have been well stocked with various species and maintained until spring, supply interesting and instructive examples of the final transformations of larvæ of insects, and especially of the nymphs of dragon-fly. The artificial warmth of the room hastens the changes so that they occur earlier than in the ponds. As the time for coming out of the larval skin approaches, many of the animals exhibit such marked changes in appearance and action that one has only to watch them closely for a few minutes to be rewarded with an exhibition of the entire process of ecdysis.

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University of Chicago.

## The Museum.

### VIII.

#### THE HALL.—Continued.

In showing the contrasted construction and contrasted appearance of ceilings with girders and ceilings with columns, the two views (Figs. 30 and 31) of the mineral hall and the geological hall of the American Museum are instructive. Naturally, the advantages of the former, as can be claimed for it by its advocates, are the free space and the absence of dust-collecting surfaces. On the other hand it is clumsy in appearance, meagre in detail, expensive, and not as safe as column construction. The column effect in Fig. 31 is decidedly graceful, and the



FIG. 30.—Present Mineral Hall, New York Museum, showing girders, without pillars.

waved ceiling an unquestionably pleasing feature. The use of columns necessitates, for artistic reasons, some cornice construction which, if elaborate, is a dust collector. This is a real objection, and it must be a matter of selection to the museum builder whether the bare, dustless ceiling is preferable to the attractive vista formed by columns in a hall, which if plafonded by cornice squares or short arches (Fig. 31) does sensibly gather dust, and to that degree implies labor and expense. The really attractive arrangement of columns in the mineralogical hall of the Natural History Museum in London (Fig. 32) would, I think, be missed if replaced by a hall without them. If columns are so built as to accommodate the cases they can not be regarded as obstructive. This is the expression of a personal preference. It must be so regarded. Certainly columns lose their architectural charm if they rise to a flat ceiling and are not connected by cornice



FIG. 31.—Present Geological Hall, showing columns (supported ceiling).

mouldings, or are not finished effectively, themselves, with pleasing capitals. At this point museum needs and architectural aims certainly should not clash, and a hall can be given a delightful structural beauty without diminishing its practical utility as a place for exhibition. Both the architect and the curator may permit themselves to meet here upon a plane of mutual forbearance and amity, if not of positive mutual admiration.

The question of windows is a critical point of adjustment. They must be so placed and of such size as to illuminate the hall, while not taking away too much useful wall space, and their exterior treatment, although affording an obvious temptation to the architect, must not become so elaborated as to diminish *at all* their necessary functions.

There are, excluding skylight constructions, three stages of lighting a hall,



(1) lighting from one side, (2) lighting from both or all sides, (3) lighting from both or all sides without reservation of wall space. Taking the first, it is evident that if the hall is wide the windows should be high and frequent, in order that the opposite wall may be reached. They should, in a hall sixty feet wide, extend almost to the ceiling, and their ratio of aggregate width to the remaining wall space, on the same side, be little or no less than one-half. In narrower halls their height can be diminished, but the ratio of window and wall space should undergo but a slight reduction. The object in view, naturally, is to allow light from the windows to reach perhaps two-thirds of the way to the opposite cases in the middle of the forenoon and in the middle of the afternoon in winter, and so far at noon, taking the summer altitude of the sun, as by reflection from



FIG. 32.—Mineralogical Hall, Natural History Museum, London.

the floor, and general diffusion, to fully illuminate them. In this respect, viz., when one side of the hall is pierced with windows, north and south halls are less advantageously conditioned than east and west halls, and of east and west halls it is also obvious that those facing south are better off for light under this condition than those facing north, it being understood that the *face* side carries the windows.

The unpleasant effect, if some should so regard it, of having tall windows can be sensibly improved by dividing the window apertures into a long lower section, and a short upper section, with transom-like partition between both. The width and number of windows and their disposition is a subject of choice, but a safe rule is to have few and broad windows, rather than many and narrow windows. Windows should not be less than five feet wide.

The second stage of lighting halls, when the lighting is from both or all sides, is the most adequate, and presents a less trying problem. As before, the windows should be high, though not of necessity as high as in the first instance, and they should be wide. In this case north and south halls have an advantage over east and west halls. Considering a four-storied simple rectangular structure as the form contemplated of the museum, the proportions of the first section of the American Museum of Natural History in New York are excellent. They can be always safely copied (Fig. 33):

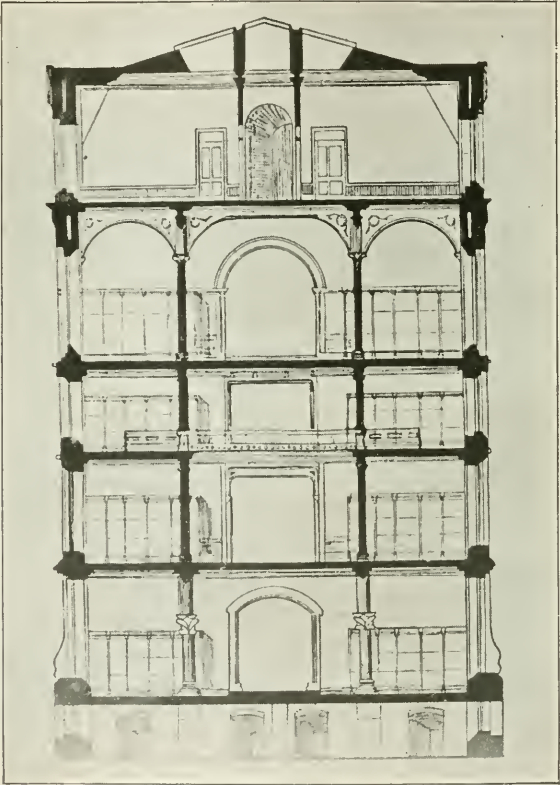


FIG. 33.—Section of North Wing, New York Museum.

Whole length of building	- - - - -	210 feet
Tower at north end holding stairway	- - - - -	20 "
Width of building	- - - - -	64 "
Height of building	- - - - -	100 "
Basement floor, above cellar, height	- - - - -	22 "
Main floor	- - - - -	25 "
Gallery floor, surrounding main floor, width	- - - - -	15 "
Gallery floor, surrounding main floor, height	- - - - -	15 "
Fourth floor, height	- - - - -	22 "
Fifth floor (work rooms), height	- - - - -	15 "

Windows on each floor, nine, opposite :

Basement, height,  $14\frac{1}{2}$  feet ; width,  $6\frac{1}{2}$  feet ; rectangular.

Main floor, height,  $12\frac{1}{2}$  feet ; width,  $6\frac{1}{2}$  feet ; rectangular.

Gallery floor, height, 8 feet ; width,  $6\frac{1}{2}$  feet ; rectangular.

Fourth floor, height, 14 feet at center ; width,  $6\frac{1}{2}$  feet ; pointed arch.

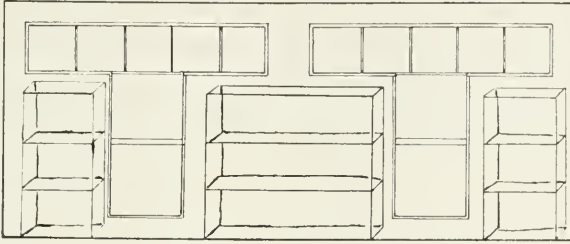


FIG. 34.—Cornice and Floor Windows.

This original section of the New York museum was placed north and south, with double illumination. Such illumination as this produced was unexcelled. There was some slight loss of wall space, but with pier cases, reaching out into the hall, this was compensated, and in the instance of flat table cases the loss of wall space is unimportant.



FIG. 35.—Successful Window Construction, New York Museum.

The third stage of lighting halls, when the lighting is from both sides, with no reservation of wall space, affords the most simple problem of all. In such halls the objects are exhibited in the center in table cases or in large upright cases, and the light can be produced most easily.

I have alluded to the device of upper cornice windows, as in the Metropolitan Art Museum of New York, and the almost entire reservation of the wall space below them. This furnishes fair results with large objects, but cannot be unreservedly recommended. Its best adaptation is secured in narrow halls. A modified form of cornice windows, introducing the floor window, is finally suggested, though I know of no illustration<sup>7</sup> of it in practice. It is given in Fig. 34.

The requisition of light, of course, varies greatly according to the nature of the objects exhibited, and large objects do not demand the same exhaustive illumination as smaller objects. There are necessarily parts of a museum that the exigencies of construction keep darker than other parts, but the exacting postulate for light should never be remitted. It is always possible to restrain or



FIG. 36.—London Art Museum.

qualify it, if in excess, and for all purposes of popular instruction and enjoyment its abundance will be found to be admired and appreciated.

Finally, in this connection, a word may again be ventured upon the outside treatment of windows. As architectural features they do offer opportunities for attractive architectural elaboration. They can be made deep set, with sloping colonades of small columns on either side, or they can be flanked by larger columns, or mullioned, or if arched, decorated with wheels in the arch, and a *marqueterie* of small panes. All such interferences with the lighting capabilities of a window should be resented, and the requisite or desirable variation in outline be limited to stone transoms and arched summits. Simple rectangular windows brought out, fairly near flush, with the outer wall, should be insisted on,



and the architectural features be repressed to the limit of some sort of exterior comeliness.

Fig. 35 shows a permissible decoration of windows without any perceptible or serious interruption of their functions. It is the north side of a west wing of the New York museum.

The question of stained glass windows in museums may also be alluded to here. There seems no reason why stained glass should not be used in museums where its use, incontrovertibly, does not mar exhibits, or in positions where its use is purely decorative and symbolic. Art museums might make very free use of stained glass in stairways and entrances, and, while an expensive embellishment, its beautiful effects would lend themselves appropriately to the æsthetic purposes of an art museum.

In view of the very general use of top-lighting in art museums, a wider latitude is permitted to the architect in the treatment of the exterior walls, and, as the objects are often of large size (sculpture, antiquities, etc.), where side lighting is adopted, the windows may be advanced to a very considerable importance in the purely architectural conception of the buildings, or their natural purposes be somewhat veiled by the construction of a peristyle, as in the very impressive façade of the London Art Museum (Fig. 36).

American Museum of Natural History.

L. P. GRATACAP.

## A Review of the Methods of Staining Blood.

### X.

#### IV. THE TECHNIQUE BEST ADAPTED TO SPECIAL PURPOSES.

In staining the blood, as in staining other tissues, the simplest method that will clearly differentiate the required structure is the best. No advantage is gained by the use of a double or triple stain when a single stain will accomplish the required result, and they may mask the feebler reactions of the primary stain. But a contrast stain is sometimes needed to ease the strain of the eyes in making differential counts. It is often advisable to first stain with a neutral compound dye and then confirm such of the elements of the blood as may be necessary with simple dyes. And the elementary student of the blood and the pathologist wish the complete structure of the blood exposed before them; the student, that he may see the different components of the blood and their relation; the pathologist, that he may see any abnormal changes in the blood.

1. **For Differentiating the Structure of the Blood.**—The neutral eosin-methylen blue dyes (III, D, 5)<sup>1</sup> stain differentially all of the histological elements of normal and pathological blood. Fixation is either accomplished in the process of staining or is described in connection with the method of staining.

Ehrlich's tricolor stain and its modifications (III, D, 4) stain all of the normal

<sup>1</sup> References are to sections where the methods are described.

elements of the blood except the granules of the mast cells and the blood plates. But slight fixation is required and dry heat (II, B, 1) is preferable, because it better preserves the granules of the leucocytes.

2. **For Staining the Red Corpuscles.**—The hemoglobin of the mature red corpuscle is yellow in color and acidophile in reaction, and is stained by the most acid dyes (III, B,). Preparations should be well fixed by dry heat (II, B, 1).

*Löwit*, for the differentiation of hemoglobin, fixed preparations in alcohol and stained them 10 to 60 seconds in an alcoholic solution of aurentia, washed in alcohol, cleared and mounted.

3. **For Studying the Development of Red Corpuscles.**—Make dry preparations from the red bone marrow by the method of Smith (I, 2,), fix the preparations in alcohol and ether (II, B, 2, b), and stain with methylen blue (III, A, 4). The cytoplasm of the immature red corpuscles is stained blue with methylen blue with an intensity inversely proportional to the degree of hemoglobin development. The nuclei of the nucleated forms are stained a deeper blue than the cytoplasm.

Aurentia and methylen blue (III, C, 1, b), was used by Bizzozero (1890) for recognizing hemoglobin in the early development of red corpuscles in birds.

4. **For Demonstrating the So-Called Degenerative Changes in Red Corpuscles.**—These changes include the polychromatophile corpuscles, the basophile granulations of red corpuscles and megaloblasts. They are mostly regenerative in nature and basophile in reaction. Löffler's alkaline methylen blue, either alone (III, A, 4, b), or counter-stained with eosin, is the simplest and best staining for this purpose. The neutral eosin-methylen blue stains also show these forms.

*Castellino* (1892) used aurentia and methylen blue (III, C, 1, c) for studying the degenerative changes in red corpuscles.

5. **For Staining Lymphocytes.**—Both nuclei and cytoplasm of lymphocytes are basophile, the nuclei strongly and the cytoplasm more feebly basophile. Fixation in alcohol and ether (II, B, 2, b) and staining with Löffler's alkaline methylen blue (III, A, 4, b) is best for this purpose.

Ehrlich recommends double basic staining with methyl green and fuchsin (III, A, 6) for demonstrating lymphatic leukæmia.

6. **For Differentiating the Granuliferous Leucocytes.**—The eosin-methylen blue neutral stains (III, D, 5) stain differentially the basophile, acidophile and neutrophile granules of the leucocytes, both of the normal polymorphonuclear forms and of the mononuclear forms (myelocytes) that appear in the circulating blood in disease.

Ehrlich's tricolor neutral stain and its modifications (III, D, 4), his neutral fuchsin-methylen blue stain (III, D, 1) and Rosenberger's phloxin-methylen blue stain (III, D, 2) differentiate the acidophile and neutrophile granules. The acidophile granules are also stained by most acid dyes (III, B).

The basophile granules of the mast cells are stained by the neutral eosin-methylen blue stains (III, D, 5) and by the dahlia solution of Ehrlich (III, A, 1, a), Westphal (III, A, 2) and Goldhorn (III, A, 1, b).

7. **For Studying the Development of the Leucocytes.**—Make dry preparations

of the red bone marrow by the method of Smith (I, 2) and stain with the appropriate stains mentioned in the next preceding section.

8. **For Preserving and Staining the Finer Structure of the Nuclei.**—*Uskew* (1890) lays great stress upon instantaneous drying of blood films for the preservation of the finer structure of the nucleus, the most minute chromatin network being preserved. Blood films made very thin and waved in the air to hasten drying would probably accomplish the desired result. Some of the methods of fixing fresh undried blood (II, A) might possibly give even better results.

Hæmatoxylin is undoubtedly the best nuclear stain for bringing out the finer structure. Either Ehrlich's hæmatoxylin and eosin (III, C, 2), Delafield's hæmatoxylin, Mayer's hæmalum (III, A, 3, b) or Mannaberg's alum hæmatoxylin (III, A, 3, a) is recommended.

9. **For Fixing and Staining the Blood Plates.**—*Hlava* (1883) used methyl eosin and gentian violet (III, C, 5) for demonstrating blood plates in preparation heated over the flame or fixed in concentrated sublimate solution or alcohol.

*Schimmelbusch* (1886) also made use of the dry method for studying blood plates. The dry intact plates are said to stain with concentrated watery or alcoholic solutions of methyl violet, fuchsin, anilin green, etc.

*Rabl* (1896) stained the blood plates with iron hæmatoxylin of E. Haidenhain (for the demonstration of centrosomes).

*Bodie and Russell* (1897) used a sodium chloride solution with a dahlia stain to prevent clumping of the blood plates.

Their formula is,—

Dahlia-glycerin	-	-	-	-	-	} equal parts.
2 per cent. sodium chloride	-	-	-	-	-	

*Ehrlich* (1900) recommends the iodine-eosin test for glycogen in the blood (see Ehrlich and Lazarus, *Histology of the Blood*) which differentiates the blood plates.

*Rosin* fixed the dry preparations for 20 minutes in osmic acid vapor, and then stained with a concentrated watery solution of methylen blue.

The polychrome neutral stains of Jenner (III, D, 5, l), Leishman (III, D, 5, m), Goldhorn (III, D, 5, o) and Wright (III, D, 5, q) and the successive staining with eosin and methylen blue after the method of Engel (III, D, 5, g), Japha (III, D, 5, h) and Goldhorn (III, D, 5, i) preserve and stain the blood plates.

*Deetjen* (1901) demonstrated the nucleated condition of blood plates. He believes that the failure of previous investigators to discover this is due to over fixation. Preparations fixed sufficiently for the preservation of the hemoglobin are over fixed for this purpose. He fixes in alcohol followed by formalin (II, B, 2, c) and stains with Ehrlich's (III, C, 2, a) or Delafield's hæmatoxylin (III, A, 3). The nuclei of the plates are stained clear blue; double stain with eosin shows a protoplasmic zone. The nuclei are still more apparent, since the protoplasm is more expanded, in preparations spread upon agar to prevent the thin blood film from drying before fixatives can be applied. This is accomplished as follows: In a 1 per cent. solution of agar filtered, is dissolved 0.6 per cent. of sodium chloride, 0.6 per cent. of sodium metaphosphate and about

0.3 per cent. of di-sodium phosphate ( $K_2HPO_4$ ). A drop of this solution is allowed to flow over a slide and cool. Fresh blood is spread on this cooled layer and covered with a cover-glass. The blood elements including the plates can then be observed alive under the microscope. Fixation is accomplished by fumes of osmic acid, by the direct application of 1 per cent. osmic acid or by Flemming's solution (II, A, 4). The fluid is allowed to flow under the cover-glass, and at the end of five minutes the cover-glass is removed and stain applied. Any (basic?) anilin dye gives good results, but hæmatoxylin alone or with eosin is more permanent. This process shows the blood plates to contain a nucleus with chromatin, sometimes in the form of a skein. This is best shown by fixing with Flemming's solution and staining with methylen blue or Heidenhain's iron hæmatoxylin.

*Dekhuizen* (1901) used 3 to 1 or 9 to 1 osmic and acetic acid (II, A, 9) containing  $\frac{1}{8}$  per cent. methylen blue, and, if desired, a trace of acid fuchsin, for fixing and staining blood plates. With invertebrates the acetic acid sometimes produces an objectionable precipitate of granular albumen; then osmic acid alone is used. Acetic acid has the objection that it decolorizes hemoglobin; with osmic acid this may with care be prevented. Osmic acid (9 to 1) cooled on ice is considered particularly favorable for the demonstration of blood plates in the blood of man and mammals. The pricked finger or ear is vigorously stirred in the cold osmic-acetic. *Dekhuizen* like *Deetjen* finds the blood plates to be nucleated.

*Argutinsky* (1901) recommends sublimate alcohol (II, B, 2, j) followed by eosin-soda-methylen blue staining (III, D, 5, k) for demonstrating blood plates. Well preserved plates are an intense red-violet, sharply outlined with a central part and an outer pale, clear blue border.

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Massachusetts State Board of Health.

## Bacteriology for High Schools.

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### IV.

#### MICROSCOPICAL EXAMINATION OF BACTERIA—Continued.

*Examination of Living Bacteria.* A slide may be prepared for this purpose by cutting a piece of blotting paper one inch square, and in the center cut a circular opening one-half inch in diameter. Place the piece of blotting paper on a slide and moisten well with water. On another slide place a large drop of water. From one of the colonies on the potato transfer to the drop a small amount of the growth; mix well. On a cover glass place a very small drop of this inoculated water. This drop should not be larger than the head of a pin. The glass slide having the paper ring is inverted and lowered over the cover glass, enclosing the drop. With a careful, quick movement the preparation is brought right side up (Fig. 15).

The diaphragm of the microscope should be nearly closed. Find the edge



of the drop with the low power and without moving the slide, place the higher power, one-sixth inch, in position. Open the diaphragm slightly and focus without moving the slide, until the edge of the drop appears as a sharp line. The bacteria should be readily detected. If not it is probably because the light is not right. Adjust this by slightly opening or closing the diaphragm.

Make "hanging drop" preparations from different colonies on the potato cultures. Also from the hay infusion.

Note the ability of certain forms to move from place to place. Many of them will exhibit characteristic "gaits." This power of independent movement is due to certain hair like appendages known as *flagella* which are distributed in various ways on the different forms. Some of the more common modes of distribution

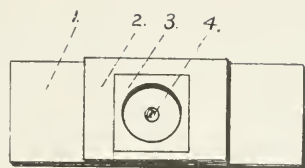


FIG. 15.—Slide arranged for making a hanging drop preparation. 1, glass slide; 2, block of blotting or filter paper; 3, cover-glass; 4, drop of water containing bacteria to be examined.



FIG. 16.—Diagram illustrating flagellated bacteria.

are shown in Fig. 16. These flagella are only visible when they are stained, and the method of staining is one of the most difficult of bacteriological procedures.

Forms which are incapable of progressive movement show a vibratory movement known from the discoverer as the *Brownian movement*, a property common to all finely divided matter in suspension.

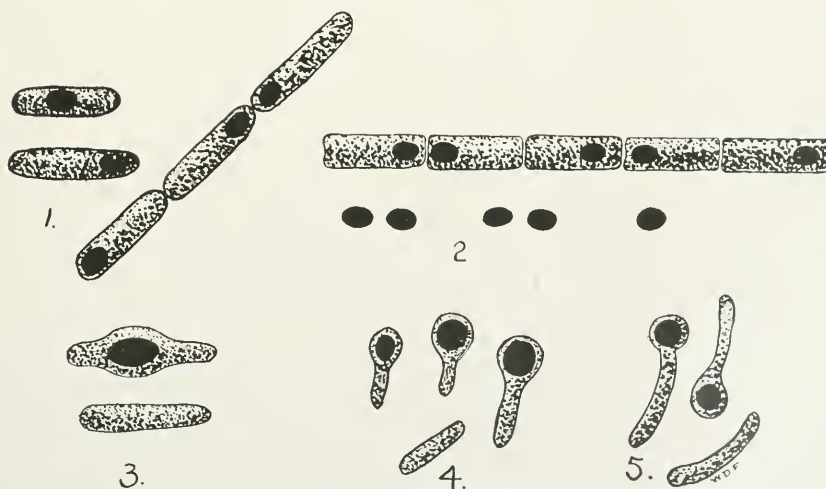


FIG. 17.—Diagram illustrating spore formation among bacteria.

The potato inoculated with dirt from the surface of a potato will show colonies with a wrinkled surface, the so-called potato bacillus. The preparation from these colonies will show rod shaped organisms. In some of the cells may be seen bright bodies which are not stained (Fig. 17). These are spores, the stage of the organism which renders many bacteria so resistant to heat, drying, and disinfectants. Note the size of the spores, whether of a greater diameter than the cell or not, and the position in the cell.

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## Methods in Plant Physiology.

### XIII.

#### HELIOtropISM—Continued.

10. **Diaheliotropism, or Transverse Heliotropism.**—The movements studied under this head are those reactions by which a plant or plant-organ is placed at right angles to the rays of light; in any other position it is out of equilibrium. The best examples are to be found in leaves. Observe plants with both fixed and motile diaheliotropic leaves, noting in each case how the diaheliotropic position is attained.

11. **Positions Due to Specific Sensitiveness.**—Find out of doors a young dandelion (*Taraxacum officinale*) or thistle (*Cnicus lanceolatus*) with leaves closely appressed to the ground. Carefully remove the earth beneath the leaves with a knife and observe whether the leaves change their position. Determine whether the change of position is forcible or passive.

Dig up a similar plant with a large ball of earth, place it in a pot and set it in a dark chamber. After 24 to 48 hours observe the change in the position of the leaves. When the leaves are about 45° above the horizontal, fasten it to the klinostat and revolve it before the window. The axis of the klinostat should point toward the window and the axis of the plant should be parallel to the axis of the klinostat. In two or three days the leaves will have curved away from the light and their laminae will be approximately at right angles to the direction of the incident rays.

Revolve another plant on the same klinostat with its axis at right angles to the axis of the klinostat and at right angles to the light rays. The leaves pointing towards the light curve downwards, while those pointing away from the light curve upwards, until the laminae of both are at right angles to the incident light.

As a result of these observations determine what stimuli are shown to be active in determining the position of these leaves.

12. **Comparative Intensity of Response to Light and to Gravitation.**—Select two crocks of mustard (*Sinapis alba*) or radish (*Raphanus sativus*) seedlings with erect stems 3 to 5 cm. high and place them in a heliotropic chamber with an opening which admits light at the level of the seedlings. Lay one crock on its side with the stems pointing horizontally toward the light, and set the other crock

erect. During the night the stimulus must be continued by the use of an incandescent gas or electric lamp. Make observations 24 to 48 hours after the beginning of the experiment, referring the behavior of the plants to geotropism and heliotropism.

13. **Epinasty and Geotropism.**—Gather some immature leaves of the dandelion (*Taraxacum officinale*) and without allowing them to wilt, embed the proximal end of each leaf in an embankment of wet sand in a tray. Only the basal 2 or 3 cm. of each leaf should be embedded in the sand. Place some leaves in the normal position and some in the reversed position. From other leaves cut out the mid-ribs and insert the proximal end of each in the sand, some in their normal position, some turned upside down. The preparations should be covered with bell-jars lined with moist filter paper and kept in the dark. Observations are to be made 35 to 60 hours after the beginning of the experiment. Determine on what environment the difference in behavior depends.



FIG. 18.—Influence of Light on the Straightening of the Hypocotyl. The lupine seedlings on the right were grown in diffuse light; the other set, planted at the same time, were grown in the dark.

14. **Influence of Light on the Straightening of the Hypocotyl.**—In two crocks of earth plant seeds of the lupine (*Lupinus albus*) placing one crock in the light and the other in the dark. When the seedlings have grown above the earth observe their behavior with regard to the curve of the hypocotyl (Fig. 18).

#### OTHER PLANT MOVEMENTS INFLUENCED BY LIGHT.

1. **Paraheliotropism.**—(a) *In chloroplasts.* Mount a leaf of *Mnium* on a slide in tap water, supporting the cover-glass with capillary glass rods. During the course of the experiment replenish as often as necessary the water lost by evaporation from the edge of the cover-glass. Clamp the slide to the stage of a microscope and set in the dark room for 30 minutes. When the microscope is removed from the darkness, examine the cells of the leaf immediately for the position of the chloroplasts. Next set the microscope in diffuse light and examine the leaf at the end of 30 minutes. Lastly place the microscope in direct sunlight and note the position taken by the chloroplasts.

Repeat the experiment, using some filaments of *Mesocarpus*, exposing them

to stimulation for 15 minutes and using the light from the mirror of the microscope each time. The chlorophyll band, which presents its broad surface to diffuse light, presents only the edge to strong light.

(b) *In plant organs.* The leaves of many common plants, particularly those of the Leguminosæ, assume positions in bright sunlight different from those taken in diffuse light. Examine the leaves of *Baptisia*, *Lonicera*, *Oxalis*, *Cassia*, or *Phaseolus* to determine at what time in the day the paraheliotropic position is assumed, the different leaf positions on the different parts of the plant, the means by which the paraheliotropic position is acquired, and the time of day when the paraheliotropic position is relinquished. Observe how the leaves behave themselves on a cloudy day.

2. **Immediate Effect of Light upon Movement.**—Find, out of doors, a dandelion in blossom. Cover the plant with a flower-crock, or other suitable object which will exclude all light. Observe the action of the flower at intervals of 30 minutes until fully closed, record the latent period and the temperature. Subsequently remove the cover and observe the time of opening.

When one plant is covered with a double-walled bell-jar containing a solution of potassium bichromate, and another with one containing a solution of ammoniacal copper sulphate, it will be seen that the orange light acts as a dark screen while the blue light acts as daylight.

3. **Nyctitropic Movements.**—The alternation of day and night sets up a series of reactions quite similar to those obtained in the preceding experiment and are known as nyctitropic movements. Study, out of doors, the sensitive plant (*Mimosa pudica*), clover (*Trifolium repens*), oxalis (*Oxalis acetosella*), or *Cassia nyctitans* to determine the time in the evening when the leaves close or fall and the time in the morning when they unfold. These observations are to be made with reference to sunshine and temperature.

By covering individual plants observe what effect darkness has on the closing of leaves at night and their opening in the morning.

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HOWARD S. REED.

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Willbrand recommends a slight departure from the usual eosin-methylen blue method of staining blood specimens. To the following solution, 50 per cent. solution of eosin in 70 per cent. alcohol, equal quantity of concentrated watery solution of methylen blue for each 50 ccm., add 10 to 15 drops of a one per cent. acetic acid solution. The preparations are placed in this solution and warmed until gas bubbles come off. The erythrocytes are stained red, the nuclei dark blue, the neutrophile granules violet, the acidophiles red (eosinophiles), and the mast cell granules intense blue. The technique is simple, and results are good. Care should be exercised in the heating of the specimens, however, as artefacts are liable to be formed, and may prove misleading —*Interstate Medical Journal*.



## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

**Cannon, Wm. A.** Studies in Plant Hybrids. The Spermatogenesis of Hybrid Cotton. Bull. Torrey Bot. Club, **30**: 133-172, pls. 7-8, 1903.

The hybrid cotton used in this investigation was obtained by crossing *Gossypium barbadense*  $\times$  *G. herbaceum*. The two mitotic divisions by which the microspore mother-cell gives rise to the four microspores are described in considerable detail. In practically all anthers of the hybrid cotton, both normal and abnormal conditions are present. In normal microspore mother-cells the first nuclear division is heterotypic and the second homotypic, and the two divisions are the exact homologues of these divisions in pure races of plants. The two sizes of chromatin ring or loops found in hybrid pigeons and in some pure forms in plants were not found in the hybrid cotton. The behavior of the chromatin at the first division could not be traced with sufficient accuracy to determine whether the two daughter nuclei were of pure or of mixed descent. If paternal and maternal chromatin is segregated as a result of the two mitoses in the microspore mother-cells so as to form nuclei with unisexual chromatin, such an organization of the chromatin would constitute a morphological basis for variation in accord with the Mendelian laws.

Many abnormal cells were observed in the anthers, but these degenerate before the first division of the microspore mother-cell. Amitosis is not rare and is probably a factor which leads to infertility. A few mother-cells showed two spindles, as described by other writers on hybrids, but in the hybrid cotton such cells degenerate before the first division is completed.

C. J. C.

**Oliver, F. W., and Scott, D. H.** On *Lagenostoma Lomaxi*, the seed of *Lyginodendron*.

Botanists have for some time been interested in the Cycadofilices, a fossil group intermediate between the Ferns and Cycads, but all evidence hitherto has been from the habit and anatomical structure of stem, root and leaf. The discovery of seeds belonging in all probability to *Lyginodendron*, a prominent genus of the group, supports the conclusions already drawn from anatomical characters. Botanists must be prepared to find that many of the plants classed under Cycadofilices possessed seeds, and that many of the "fern fronds" of paleobotanists belong rather to Spermatophytes.

C. J. C.

**Guignard, L.** La formation et le développement de l'embryon chez l'*Hypecoum*. Journal de Botanique, **17**: 33-44, figs. 1-20, 1903.

The development of the embryo of *Hypecoum*, one of the Papaveraceæ, is very peculiar. After the second division of the fertilized egg, the structure looks like a two celled embryo with a unicellular suspensor, but the three cells become separated at this stage, and one of the cells of the apparent embryo gives rise to the entire embryo proper. The other two cells never divide, but become extremely large, the nucleus alone of

one of these cells being larger than an entire embryo at the ten celled stage. Double fertilization was not actually observed, but there are indications that it occurs.

C. J. C.

**Guignard, L.** La double fécondation chez les Crucifères. Jour. de Botanique, 16: 361-368, figs. 20, 1903.

In a recent study of *Capsella Bursa-pastoris* and *Lepidium sativum* Prof. Guignard reports "double fertilization"

in both forms. In *Capsella* the fusion of the polar nuclei is very late. At the time of fertilization the antipodals are much reduced and soon disappear. The male cells while in the pollen tube are closely applied to each other and are either ovoid or slightly elongated. A delicate faintly staining envelope of cytoplasm surrounding each nucleus could be observed with great difficulty. This is extremely interesting, as it seems to show that fertilization may be not merely a union of nuclei but also a fusion of cytoplasm with cytoplasm.

In *Lepidium sativum* the male cells are fully formed in the pollen grain. As in *Capsella*, they remain close together until discharged into the embryo-sac.

Chicago.

W. J. G. LAND.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole, 55 S. Marengo Avenue, Pasadena, Cal.

**Ancl, P.** Sex Determination of Gametes in Hermaphrodite Gonads. Arch. Zoöl. Expér. 10 (Notes et Revue), 84-94, 1902 (Review in Journ. of Royal Micr. Soc., Feb., 1903).

The author maintains, with especial reference to *Helix pomatia*, that the sex-cells are at first indifferent. That those which appear *before* the nutritive

elements are formed become male cells, giving origin to spermatozoa, and that those which appear after the appearance of the nutritive elements become ova. This conclusion the author thinks may be extended to similar cases; the "cyto-sexual" character of the gametes is thus determined by the time of their appearance in relation to the appearance of the nutritive cells. (This is an interesting confirmation of the general results pointing to the greater quantity of nutrition required for producing ova.)

A. M. C.

**Scriven, J. B.** Preparing Serial Sections of Insects. Journ. Quekett Micr. Club, 8: 343-348, 1902. (Rev. Journ. of Roy. Micr. Soc., Feb., 1906, 1903.)

The technique of Lowne is generally followed, but several time-saving modifications are introduced. After fixa-

tion the object is dehydrated in *hot* absolute alcohol and put at once into the following embedding medium: Paraffin (45° C.) 80 grs., white wax 10 grs., anhydrous creosote 2 minims, solution of caoutchouc in pure benzol (1 gr. to 5 fluid drs.) 2 minims. This medium cuts well at the temperature of the room (about 16° C.). The sections are stretched on and fixed to the slide with warm water. After drying by evaporation the embedding medium is removed by a rapid flooding with benzoline, which in its turn is removed with absolute alcohol. Other steps are those usually adopted.

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

Slonaker, J. R. The Eye of the Common Mole, *Scalops aquaticus machrinus*. Jour. Comp. Neur. 12: 335-366, pls. 18-20, 1902.

The eye was either fixed and hardened *in situ*, or, if removed, the orientation was maintained by attaching it to a slip of paper until it was ready to be embedded. Perenyi's fluid preserved the shape of the eye better than other fixing agents, but gave indifferent histological detail. This was better preserved in 10 per cent. formalin or in potassium bichromate. Saturated solution of bichloride of mercury, absolute alcohol, 10 per cent. nitric acid, 50 per cent. alcohol, and platino-aceto-osmic mixture did not give satisfactory results. The potassium bichromate usually caused the retina to separate from the choroid and pigment layers. Sections were stained on the slide in Ehrlich-Biondi, in Weigert's or Minot's hæmatoxylin and in hæmalum.

C. A. K.

Child, C. M. Regeneration of the Appendages in Nymphs of the Agrionidæ. Arch. f. Entwicklungsmechanik. 15: 543-602, pls. 20-22, 1903.

Nymphs were obtained at all seasons of the year in shallow ponds and swamps in the vicinity of Chicago. They were kept in Stender dishes or tumblers with a spray of *Elodea* to provide oxygen, and in addition the water was aerated once in two or three days. It was necessary to isolate the individuals in order to make record of results, and to prevent mutilation. To provide for the emerging imago small sticks were placed in the aquarium reaching above the level of the water and the dish was covered with gauze. The nymphs were fed upon planarians, larvæ of gnats and small amphipods. Sudden variations in temperature were often fatal. Growth was most rapid at summer heat. Alcohol gradually diffused through the aquarium did not produce quiescence in the nymphs until the second day and they rarely survived the treatment. For purposes of operation it was found admirable to place the nymph on a piece of blotting paper on the stage of the dissecting microscope and to add a drop of chloroform to the margin of the paper. Anæsthia followed immediately and the nymphs recovered after the operation.

C. A. K.

Neuhaus, C. Die postembryonale Entwicklung der Rhabditis nigrovirens. Jena. Zeitschr. 37: 653-690, Taf. 30-32, 1903.

This Anguillulid nematode was obtained from the lungs of *Rana temporaria*, where it is found in association with *Distomum cylindraceum*. Both lungs are usually infected and often contain as many as 25 of the nematode worms. Great difficulty was encountered in the fixing of the ova on account of the exceedingly resistant egg shell. The following method was finally hit upon as the most effective. The worms were fixed for 24 hours in Boveri's picro-acetic after cutting off both ends to permit ready access of the fixing fluid. On 5 $\mu$  sections superb nuclear and yolk differ-

entiation was obtained by double staining with hæmatoxylin and orange G. Cell boundaries were clearly defined by double staining in hæmatoxylin and alum carmine. Total preparations for control of sectioned material were made by dissecting out the eggs from the worms, fixing in picro-acetic, washing out in alcohol, and then staining in a mixture of pure glycerine and enough acetic carmine to give a pale rosy tint to the mixture. In 1 to 2 days the eggs become very transparent and give splendid optical sections in any desired plane when mounted under covers on hairs for rollers. This stain is not permanent.

For the study of development after the hatching that normally takes place in the intestine or fæces of the frog, the method of Leuckart was used, but in modified form to insure pure cultures. The culture medium, a mixture of intestinal contents and earth, was first sterilized by heat and then planted with embryos which were removed from the parent by dissection.

C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory,  
University of Michigan, Ann Arbor, Mich.

Stade, W. Untersuchungen über das fettspaltende Ferment des Magens. Beitr. z. chem. Physiol. u. Path. 3: 291-321, 1902.

In the course of this study of the fat splitting enzyme of the stomach, steapsin, the author discovered a new and

interesting law of ferment action. The method of experimentation followed was to add to a definite amount of fresh gastric juice a known quantity of egg yolk. Portions of the yolk were removed after digestion had proceeded for definite periods of time, and the amount of fatty acid which had been split off determined by analysis. It was found that the percentage of fatty acid formed after different times of digestion were very nearly equal to the square roots of the times of digestion. This relation is shown by the following results from a single series of experiments:

1.95	2.8	3.4	3.8	4.8	6.85	per cent. of fatty acid
after 3	6	9	12	24	48	hours digestion.

The square roots of the times of digestion are

1.7	2.5	3.0	3.5	4.9	6.9
-----	-----	-----	-----	-----	-----

The agreement is seen to be very close. This law may be generalized in the following formula:

$p : p_1 : p_2 = \sqrt{t} : \sqrt{t_1} : \sqrt{t_2}$ , in which  $p$  stands for percentage of product formed and  $t$  for time of digestion.

R. P.

Seligman, C. G. On the Physiological Action of the Kenyah Dart Poison Ipoh, and its Active Principle Antiarin. Jour. Physiol. 29: 39-57, 1903.

The Kenyah dart poison is prepared by the natives by heating the sap of the upas tree, *Antiaris toxicaria*. After heating the sap slowly solidifies into a

hard, black resinoid mass, which is almost entirely soluble in water. This sub-



stance is used as the dart poison without further admixture. The active principle of the sap is a crystalline nitrogen-free glucoside having the formula  $C_{21}H_{30}O_8$  and called antiarin. The physiological action of ipoh on frogs is to produce clonic spasms of the muscles, paralysis, and systolic arrest of the ventricle. The injection of a solution of the crystallized antiarin produces the same results except that the clonic spasms are absent. With mammals and some birds the same effects are produced and in addition there are marked gastro-intestinal symptoms, e. g., profuse diarrhœa. It was found that certain birds, notably fowls and pheasants, possess a high degree of natural immunity to this poison. This is thought to be due to a greater power of resistance of the heart muscle in these cases.

R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt, Harvard University Medical School, Boston, Mass.

**Kaiserling and Orgler.** Ueber das Auftreten von Myelin in Zellen und seine Beziehung zur Fettmetamorphose. *Virchow's Archiv*, 167: 296, 1902.

The writers have discovered by means of the polariscope a double refractive substance in cells which resembles fat morphologically. They hold that it is

identical with the myelin described by Virchow. Hence this name is retained. The droplets are stained light gray by osmic acid, never coloring as intensely as fat. With sudan III or scharlach R they take a red hue. Myelin stained with osmic acid, unlike fat, is decolorized by xylol, chloroform, and oil of bergamot. In hardened tissues the double refraction of the myelin is lost. Myelin droplets were found in the intima cells of arteries in fatty metamorphosis, particularly in the aorta, in an amyloid kidney, and a great white kidney of chronic Bright's disease, in the epithelial cells of the pulmonary alveoli in pneumonia and tuberculosis and in the bronchial secretion, also in the corpus luteum of the ovary, the cortex of the adrenal and the retrogressive metamorphosis of the thymus gland.

Myelin was never demonstrable in the heart. It was likewise absent in fatty infiltration of the liver, in the normal fat-deposits of the body, in colostrum, in the secreting mammary gland and in many cases of fatty metamorphosis of the kidney.

J. H. P.

**Fischler, F.** Ueber den Fettgehalt von Niereninfarcten, zugleich ein Beitrag zur Frage der Fettdegeneration. *Virchow's Archiv*, 170: 100-151, 1902.

The following conclusions are based on a study of the fat in kidney infarcts, which were produced experimentally in rabbits.

The appearance of visible fat in cells, as well in the so-called fatty degeneration as in fatty infiltration, is dependent not alone upon a certain condition of the cell, but also upon factors external to the cell.

Some circulation must be maintained of the blood, lymph or diffusion stream, although it need not be complete, or fatty metamorphosis will not occur.

The most essential condition for the appearance of fat is the life of the cell. Dead cells do not undergo fatty change. Without doubt the presence of fat within the cell can be due to a great variety of conditions.

The experiments summarized in this study add no support to the view that fat can originate from the cell proteids. J. H. P.

**Councilman, Magrath and Brinckerhoff.** A Preliminary Communication on the Etiology of Variola. Jour. of Med. Research, 9: 372-375, 1903.

In the epithelial cells of the skin in smallpox the writers have discovered peculiar bodies which they regard as living organisms and the cause of the disease. Two cycles of development are described. The primary or *intracellular* cycle of the parasite occurs in vaccinia as well as in variola, but the second or *intranuclear* cycle, which is possibly sexual in character, takes place only in variola. The intranuclear development was also demonstrated in the monkey, and this is the only animal in which variolous lesions can be produced.

The first stage of the intracellular cycle was observed by Guarnieri in 1892, but the bodies he described have been regarded by many authorities as degeneration products. They were small structureless bodies from one to four microns in size. The writers found them in the lower layers of the cutaneous epithelium before vesicles had formed. The bodies increased in size, granules appeared and the vacuole which surrounded each parasite enlarged until a central space about the nucleus of the epithelial cell was formed. The larger bodies may exceed the size of the nucleus of the epithelial cell. They have an irregular contour and resemble amœbæ. No definite nucleus is demonstrable within the bodies. Segmentation of the parasite occurs with the formation of round spore-like structures about one micron in size.

Coincident with the segmentation and disappearance of the intracellular forms, small ring-like bodies appear in the nuclei of the epithelial cells. These intranuclear organisms enlarge and consist of a circle of spherules which surround a large central vacuole. After further development segmentation occurs. The cell containing the parasite undergoes complete degeneration. The spores formed by the intranuclear organism are considered by the writers as the true infecting material of variola. They are made out with difficulty and were first seen in a photograph of the tissue. By the time young vesicles have formed the development of the parasite is completed and only spores are present in the later lesions of the disease. J. H. P.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn, Wesleyan University, Middletown, Conn.

**Tsiklinsky.** Sur la flore microbienne thermophile du canal Intestinal de l'homme. Ann. de l'Inst. Past. **17**: 217, 1903.

In recent years it has become a matter of some interest to determine the character of the normal bacteria in the

human intestine, since many important problems related to the healthfulness of foods are associated with their bacterial content. It has been long known that the intestine commonly contains two bacilli, *B. coli communis* and *B. lactis aerogenes*, and later Tisser has shown that a strictly anaerobic bacillus called *B. bifidus* is also very common. The author of this article has extended this information by the detection in the intestine of a number of the so-called *thermophilous* bacilli. The method of work is simple and consists of inoculating agar tubes and plates with the contents of the intestine and cultivating them at high temperatures, the precaution being necessary to place the plates upside down to prevent the water that collects on the surface from distributing the bacteria and ruining the plates. The results of the work have shown: 1. There are always present in the intestine a considerable number of bacteria which will grow at high temperatures, 50° to 60°. Of these he describes 20 species, some of which grow only at such high temperatures. 2. The bacteria in the intestine differ very markedly in different localities. The study of the contents of the intestine of nursing infants in Paris and in Russia has shown a widely distinct bacteria fauna in the two cases, in which there is only one or possibly two species found in common. His conclusions are: 1. These bacteria play no important part in the chemical changes of digestion. 2. Since some of these bacteria are unable to grow in culture media except at high temperatures there must be conditions in the intestine more favorable than in culture media, because they certainly do develop in the intestinal content at a temperature below 50°. 3. Some of these thermophilous bacteria are only modified forms of ordinary bacteria.

H. W. C.

**Marmorek.** L'Unité des Streptococques pathogènes pour l'homme. Ann. de l'Inst. Past. 1902, p. 172.

The presence of streptococci is an extremely common characteristic of quite a variety of pathological conditions in

man. They are found in many forms of pus infections, in blood poisoning, in scarlet fever, and in a large variety of other abnormal conditions. The author of this work has conceived the idea of testing a large variety of such streptococcic cultures, from different sources, to determine if possible whether they are distinct species or identical. In all he tests 42 different cultures from different sources, including many different pathological conditions in man. The diagnostic characters which he uses most prominently for their distinction is the hæmolysis of rabbit blood and the impossibility of the streptococcus to grow in its own fil-

trates. A third bio-chemical character used was based upon the inoculation of anti-streptococcic serum into rabbits to determine whether the animals were thus protected against the different streptococci. The results of the experiments is somewhat surprising, for he finds that with one exception the streptococci all have the same characters, and are indistinguishable from one another. The one exception is a streptococcus found in scarlet fever which proves to have very little power of hæmolysis upon rabbit serum. The conclusion that all pathogenic streptococci are identical or belong to closely identical types is a striking one. It certainly could hardly have been anticipated beforehand, and shows the inadequacy of our present method of diagnosis. In a measure confirmatory of these results is a paper by Aronson in the Berlin Klin. Woch. Oct. 27, 1902, experimenting with streptococci from scarlet fever, diphtheria, erysipelas, and acute rheumatic fever. While his cultures at first showed different characteristics, they were lost after cultivation. Aronson also concludes as to close relationship of all species, since serum from a horse will minimize against all streptococci.

H. W. C.

**Mouton.** Rescherches sur la digestion chez les amibes et sur leur diastase intracellulaire. Ann. de l'Inst. Past. 16: 457, 1902.

That larger animals and plants serve as food for many parasitic bacteria is well understood, and it is interesting to read

of a series of observations showing an instance in which the bacteria themselves serve as the food. The author has studied amœba which he has found in garden earth. Experience has shown that it is impossible to keep amœba alive in pure cultures. But the author finds that they can be kept alive if a quantity of certain bacteria. *B. coli* being the one used here, is mixed with the amœba, the amœba feeding upon the *B. coli* and thus sustaining themselves in culture. It is the digestion of the bacteria that the author studies, and the purpose of the present experiments was to determine whether the amœba produces a proteolytic enzyme by which they digested the bacteria. The author's method of experimenting is as follows: A mixed culture of amœba and bacteria is placed in a centrifuge and rapidly rotated. The liquid is then removed and the sediment is mixed with two or three times its volume of glycerine and again centrifuged. The glycerine dissolves from the amœba the enzymes present, and the solution may be subsequently used for experimental purposes. Tests of the glycerine extract, in the presence of chloroform to prevent the growth of micro-organisms, show the presence of an enzyme which rapidly digests fibrin and other proteids, the results being quite similar to that of trypsin digestion. The conclusion would of course have been anticipated, but the method of obtaining a proteolytic enzyme from micro-organisms is new.

H. W. C.

**Weber.** Die Bakterien der sogenannten sterilisirten Milch, u. ihre Beziehungen zu den Magendarmkrankheiten der Säuglinge. Arb. a. d. Kais. Ges. A. 17, 108, 1901.

The author has made an examination of 150 samples of sterilized milk from eight different dairies in Berlin. In no dairy does he find that all samples of

milk are sterile. Eighteen species of bacteria are isolated from 150 samples. These bacteria all have the power of peptonizing casein, producing putrefactive and other injurious changes in the milk. He concludes, therefore, that the in-



complete sterilization of the milk, which destroys the acid bacteria but not the peptonizing forms, only offers a greater chance for the putrefying bacteria to grow in the milk. The lactic bacteria are, therefore, a means of protecting the milk from these injurious changes. He further tests the eighteen different species of bacteria found, and among them finds two which are distinctly poisonous, both belonging to the groups of bacteria already described by Flügge.

H. W. C.

## GENERAL LABORATORY TECHNIQUE.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory,  
University of Michigan, Ann Arbor, Mich.

### The Preservation of Anatomical Material.

A reliable and altogether satisfactory method of preserving material for dissection is a thing which has been long desired by teachers of anatomy. Keiller ("On the Preservation of Subjects for Dissection by Injection with Formalin and Carbolic Acid Solutions and Storage in Similar Solutions." Proc. Assoc. Amer. Anat. 16th Session, p. vii-viii, 1903) offers a series of solutions which he has found very satisfactory in preserving human subjects. The formulæ for these solutions and the special purpose for which each is designed are as follows:

*Formula 1.*—For section (macroscopic) and special demonstration of the viscera in situ:

Formalin	-	-	-	-	-	-	-	-	-	-	2.5
Carbolic acid	-	-	-	-	-	-	-	-	-	-	2.5
Glycerine	-	-	-	-	-	-	-	-	-	-	10.0
Water	-	-	-	-	-	-	-	-	-	q. s. to	100.0

*Formula 2.*—For ordinary dissecting purposes:

Formalin	-	-	-	-	-	-	-	-	-	-	1.5
Carbolic acid	-	-	-	-	-	-	-	-	-	-	2.5
Glycerine	-	-	-	-	-	-	-	-	-	-	10.0
Water	-	-	-	-	-	-	-	-	-	q. s. to	100.0

*Formula 3.*—For operative surgery:

Formalin	-	-	-	-	-	-	-	-	-	-	1.0
Carbolic acid	-	-	-	-	-	-	-	-	-	-	2.5
Glycerine	-	-	-	-	-	-	-	-	-	-	10.0
Water	-	-	-	-	-	-	-	-	-	q. s. to	100.0

*Formula 4.*—For storage tanks:

Formalin	-	-	-	-	-	-	-	-	-	-	1.0
Carbolic acid	-	-	-	-	-	-	-	-	-	-	2.0
Water	-	-	-	-	-	-	-	-	-	q. s. to	100.0

Glycerine to the amount of  $2\frac{1}{2}$  per cent. will be an advantage, but is not necessary.

When any of the first three of these solutions are used they are to be injected into the blood vessels through some large and accessible artery, usually the femoral. Keiller recommends that pressure injection be used, rather than the ordinary brass syringe and hand pressure, and that the pressure be maintained for a long time. In this way large quantities of the fluid may be injected. The success of the preservation depends in a large measure in getting in a large quantity of the fluid. The specimens, after injection, may be kept indefinitely on the tables without immersion. They should be kept covered with cloths, to prevent drying out. The reviewer has used solutions 2 and 4 with very satisfactory results in preserving cats for dissection. No mould forms on the specimens, as happens when formalin is used without carbolic acid for injection.

For a colored injection mass Keiller recommends the following :

*Formula 5.*—Colored injection mass :

A.	Potassium bichromate	-	-	-	-	-	-	3 ounces
	Water	-	-	-	-	-	-	1 pint
B.	Lead acetate (commercial)	-	-	-	-	-	-	6 $\frac{3}{5}$ ounces
	Water	-	-	-	-	-	-	1 pint
C.	Gelatine (commercial)	-	-	-	-	-	-	4 $\frac{1}{2}$ ounces
	Water	-	-	-	-	-	-	1 pint

Dissolve A, B, and C in separate stone jars immersed in a large fish kettle and raised very nearly to the boiling point. Strain the gelatine solution through a fine wire strainer into a vessel capable of holding two quarts; add to this, while hot, the hot bichromate solution (also strained), stir well and add gradually, while hot, the acetate of lead solution (also straining it). Inject, while hot, with a brass anatomic syringe, using about as much force as you can exercise with the hand. The fluid should be so hot that you require to protect with a cloth the hand holding the syringe. A human body requires about a pint of this mass. The specimens need not be warm and will be ready for dissection after twenty-four hours. Bodies should be injected with the color mass not less than a week after the preservative fluid has been injected.

R. P.

**A New Method of Fixing Paraffin Sections to the Slide.**

Michaelis<sup>1</sup> describes a method of fastening sections to the slide which combines the advantages of the dry albumen and the water methods. The sections are floated on the surface of warm water (ca. 45° C.) until they have straightened, and are then lifted on a clean glass slide. Drops of adherent water are removed with filter paper. Then a piece of *smooth* writing paper is pressed firmly on the sections as they lie on the slide. The sections, of course, stick to the paper. The paper should then be trimmed down with scissors until none projects beyond the edges of the paraffin of the sections. A slide is then coated with albumen fixative in the ordinary way, and the sections (attached to the paper) pressed firmly down on this coated slide. The albumen is then coagulated by heat and the paraffin melted over a flame in the ordinary manner. When the slide is dipped into xylol the paper falls to the bottom of the tube as the paraffin is dissolved.

The advantages of the method are that the sections are perfectly flat and firmly fastened to the slide. It is especially useful in handling large sections.

R. P.

<sup>1</sup> Centralbl. f. Allgem. Path. 14: 264-265, 1903.

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**SEPARATES.**

One hundred separates of each original paper accepted are furnished the author, gratis. Separates are bound in special cover with title. A greater number can be had at cost of printing the extra copies desired.

THE SEMI-CENTENNIAL OF THE OPTICAL INDUSTRY IN AMERICA.—We had the pleasure of witnessing in our city on July 24th and 25th the Golden Jubilee of what is really the anniversary of the optical industry in America, and since our readers are so much interested in the products of the company whose fiftieth anniversary was just commemorated, we have thought best to include a brief account of it.

The Bausch & Lomb Optical Company was founded in July, 1853, by the association of Mr. J. J. Bausch and Mr. Henry Lomb. At this time there was practically no optical work done in America, the optical and scientific apparatus used being brought into the country from abroad. It was of a very inferior quality, as compared with the standards of to-day, and its high price made it extremely rare to find a laboratory with any equipment of microscopes or other optical appliances: and while Bausch & Lomb did not, in the beginning, manufacture microscopes, they at once began the manufacture of lenses, and it was their continued efforts in this line which later made it possible for them to take up the manufacture of the more delicate optical instruments with which their names are so widely connected in the scientific world to-day.

The first lenses were ground on an old fashioned hand machine in the first office in the Reynolds Arcade in this city. They were spectacle lenses which were fitted into sheet horn, imported from abroad. The growth of the industry, which received its chief impetus through the high price of gold during the civil war, by which a demand for horn and later for vulcanite-framed eyeglasses was created, necessitated the taking of a factory on the corner of Andrews and Water streets in 1854. The demand for eyeglass frames set with lenses having very considerably increased, it was deemed advisable to manufacture all the lenses, instead of importing them, as previously, and a power lens-grinding and polishing machine was devised by Mr. Bausch, who thus established the first power lens-grinding plant in America. The lens-grinding facilities were gradually increased until, in 1868, it was necessary to take a larger building on the corner of River and Water streets. Here the work of perfecting the lens-grinding process was continued until 1874, when the business had outgrown the facilities at hand, and a new building was constructed on the site of the present factory, where a larger and more complete lens-grinding equipment was installed, new and improved grinding devices having been designed and built for the purpose.

It was in the year 1875 that the manufacture of microscopes was commenced under the supervision of Mr. E. Gundlach, one of the most advanced scientific opticians of his time, who remained with the company for the first two years after the beginning of the experiments in the development of microscopes. A year of hard work and experimentation was consumed in getting the first models of microscopes in a satisfactory condition. These models were exhibited at the Centennial exhibition in Philadelphia in 1876, and created a very favorable impression among the scientific men who examined them. There was a general desire among the scientific and educational workers at this time to use the microscope as an accessory in teaching biological and other sciences, but the prices at which good microscopes could be had were prohibitive; in fact, no

attempt had been made by any of those engaged in the manufacture of microscopes to produce them in large quantities, or at reasonable figures. From the outset this has been one of the main purposes of Bausch & Lomb, and on this basis they received, from the beginning of their efforts, the hearty support of men of science in all parts of the country. Even with this support, the experiments were conducted with tremendous losses for many years, and had it not been for the prosperous condition of the eyeglass and lens part of the business, the attempt would have been disastrous in the extreme. The only thing which kept the microscope department of the business going was the indomitable resolve to accomplish the purpose which had so long been cherished, to produce in America the highest quality of optical instruments, and to produce them in commercial quantities.

While the balance sheet was going down, the quality of the work was steadily improving, which, of course, began to have its effect on the sales, and when the day finally came that the books showed no actual loss, there was great rejoicing.

The introduction of American microscopes of standard quality, and at moderate prices, seemed to have a very stimulating effect on their educational and industrial uses, as well as on their use by individuals as a means of recreation and culture. Here, as in all other lines of work, the battle against adverse conditions in manufacture was less strenuous than that which it was necessary to wage against the deep seated conviction in the public mind that optical work of high excellence could be done only in Europe, that American skill and ingenuity were unable to cope successfully with the problems presented in optical manufacture.

The tide gradually turned, however, and the Bausch & Lomb products found their way over the length and breadth of the land, wherever microscopes are used. The extensive relations which were brought about through the supplying of microscopes to educational institutions, by which the most advanced thought of educational workers was directed toward the improvement of microscopes, microtomes and other laboratory apparatus which was being manufactured, demanded the addition, as a convenience to their patrons, of supplies of chemicals, chemical glassware, stains, microscopic objects, microscopical tables, cabinets, etc., until at the present time a large department is required to handle this line of goods, and it has been necessary to build a special factory in Germany for the production of chemical glassware in order that the supply may be constant, uniform in quality, and of the most approved construction. The machinery for much of the delicate work in this factory was designed and built in the machine shops of the Rochester plant, and embodies many original features which are readily seen in the quality of work being turned out in Germany.

The friendly relations which have, since the beginning of the manufacture of microscopes, existed between the company and the scientific workers in all parts of the country, have resulted in the manufacture of many kinds of apparatus which cannot be considered as strictly optical. The use of the microscope in the bacteriological laboratory, as the importance of bacteriology became appreciated, called for the supplying of incubators, sterilizers, autoclaves, etc.

The application of centrifugal force to the collection of urinary and other sediments, and to the separation of blood elements for examination under the microscope, suggested the construction of a suitable centrifugal apparatus propelled by hand, water and electric power, and these also became a part of the manufacture. So on through the list.

The growing business demanded still greater facilities, and a new building was constructed on the north side of the main factory building in 1892. The lower portion of this building, which is excavated out of the solid rock, contains vaults for the storage of the many varieties of optical glass required in the production of the various kinds of optical instruments. The general supplies for the various departments are also kept there.



One not well acquainted with the requirements of a large modern manufacturing establishment would be surprised at the number and variety of the articles required to be kept in stock in this department. A trip through it would disclose stores of hardware, cloths, metals, brushes, boxes, papers, soaps and articles too numerous to mention, sufficient to stock a large general store.

In the early stage of the business Mr. Bausch gave his personal attention to the supervision of the work and to the inspection of the finished product, but as the manufactures increased this work devolved upon the younger members of the company. The subsequent enlargement of the plant, with the increased producing power, and larger number of workmen, made it impossible for them adequately to oversee all parts of the work-shop, and it was necessary to entrust their duties to others. This involved the development of a system of supervision and inspection, which in itself is a marvel of intricacy and perfect adaptation to the purpose for which it was devised.

Each article, whether simple or complex, must not pass out of the establishment until it is fully up to the standard of quality established for it, as a defect, no matter how insignificant, whether in a special lens, a photographic objective, a searchlight, or in the optical performance of a microscope objective, will seriously affect the reputation of the company. The constant aim is to reach a higher standard of excellence, to foster a spirit of higher attainment in the productive force, and to eliminate the possibility of the production of work defective in any way. With the mass of detail connected with the production of thousands of different parts, of many different kinds of material, for an infinite variety of purposes, the assembling of them, fitting them, until they form a complete whole, requires an inflexible system in the conduct of the work-shop, an untiring vigilance of inspection at every process, and an accumulation of skill in the workmen which must be complete in every detail, in order that every article turned out may be uniform in quality with every other.

Desiring to provide some temporary means of support for employees who are thrown out of employment through sickness, and to assist the families of deceased members, the Bausch & Lomb Optical Company Mutual Benefit Association was organized April 16, 1881.

Any person having been in the employ of the company two months' time may become a member of the association. Since its establishment this society has paid out in benefits to its members over \$34,500. The membership at the present time is 930.

In 1900 a fund was established by Mr. J. J. Bausch, Mr. Henry Lomb and the company, with a desire to assist in providing a comfortable future for employees who have served the company many years.

The fund was materially increased by them on this anniversary.

The technical nature of the business and the necessity for coming in close contact with the trade made it necessary to establish a series of branch offices, and in addition to the office opened in New York in 1866, and which is now in charge of Mr. Henry Fincke, an office was established in Chicago in 1896 under the management of Dr. Wm. H. Knap, and another office was opened in Boston in 1903 under the management of Mr. L. M. Potter. These offices are conducted largely as a convenience to the trade, as the company has business relations with nearly every dealer in optical goods in America.

In 1902 a separate company under the title of the Bausch & Lomb Optical Company, G. m. b. H., was organized in Germany. This office is at Frankfurt, a/m, under the management of Mr. August H. Lomb. This company does a general merchandizing business in chemical glassware and different kinds of scientific apparatus, as well as in the Bausch & Lomb Optical Company's products, both selling in Europe and exporting to America. This company also owns the glass factory for the manufacture of chemical glassware. The demand for Bausch & Lomb products abroad has made it necessary to establish depots in

foreign countries where these products can be obtained. Accordingly an arrangement was entered into with responsible houses to carry a full line of the Bausch & Lomb manufactures in Paris; Tokio, Japan; St. Petersburg, Russia; Barcelona, Spain; London, England; City of Mexico; Toronto, Canada; and Brisbane and Sidney, Australia. A complete stock of the Bausch & Lomb photographic, microscopic and field glass products, as well as eyeglasses and lenses of various kinds, is thus available in these countries.

In addition to these houses that carry complete stocks, and are therefore supply depots for the countries, the company maintains trade relations with the leading houses dealing in optical goods everywhere.

In looking back over the record of facts in these fifty years of struggle, the things which most impress one are the innumerable difficulties which have been surmounted, the tenacity and intensity of purpose to produce better articles than have ever been produced before, regardless of the sacrifices necessary in producing them, and the determination to produce every part under one roof, in order that its quality be known and may contribute to the production of a whole of known excellence.

In commemoration of the completion of their fifty years of business, the Bausch & Lomb Optical Company tendered a complimentary entertainment to their 1200 employees, and to about one thousand invited guests in the Lyceum Theatre. The exercises were opened with an address by Hon. A. J. Rodenbeck, mayor of Rochester, in which he reviewed the history of the development of the company from its small beginning to the present time, and referred in a touching manner to the fifty years of congenial association which Mr. J. J. Bausch and Mr. Henry Lomb have enjoyed, and to the growth of the industry which has, in its own developments and in the development of the allied industries which it has made possible, made Rochester the optical center of the world.

Judge William E. Werner also made an address, reviewing the relations of the industry with the development of the optical trade, and development of optical sciences.

In addition to these addresses, a choice musical program, interspersed with monologue, dialogue and other entertaining numbers, was rendered. During the intermission, Senator W. W. Armstrong appeared on the stage and requested that Messrs. Bausch and Lomb be brought before him. When they had been escorted to his presence by two of the oldest employees of the company, Senator Armstrong uncovered three massive, golden loving cups, which he presented in the name of their 1200 employees, one to Mr. J. J. Bausch, one to Mr. Henry Lomb, and one to the Bausch & Lomb Optical Company. The cups presented to Messrs. Bausch and Lomb had etched on one panel a representation of their first humble factory, and on another the present structure. On the third panels were presentation inscriptions which read: "Presented to Mr. J. J. Bausch in commemoration of the fiftieth anniversary of his association with Mr. Henry Lomb, and of the founding of the Bausch & Lomb Optical Company, by their employees—1853-1903," that presented to Mr. Lomb being similarly engraved with the substitution of his name for that of Mr. Bausch. The cup presented to the company was engraved on one panel with the first and present factory buildings, on another with the profiles of Mr. J. J. Bausch and Mr. Henry Lomb, and on the third with a presentation inscription, which was: "Presented to the Bausch & Lomb Optical Company in commemoration of their Fiftieth Anniversary, by their employees—1853-1903."

At the door of the theatre each guest was presented with a large envelope containing a beautifully engraved and embossed announcement of the completion of fifty years of optical manufacture; a souvenir brochure containing an historical sketch of the development of the Bausch & Lomb Optical Company's manufactory from its beginning to the present time, by L. B. Elliott; and a program of the evening's exercises.

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# Journal of Applied Microscopy and Laboratory Methods

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## Staining Paraffin Sections on the Slide.

There are various methods of dealing with paraffin sections after they have been fastened to the slide. The method formerly employed in our own laboratory was as follows: The slide was heated until the paraffin melted and then the entire slide was placed in a Stender dish of xylol until the paraffin was removed. To avoid the danger of overheating, the slide was often placed in xylol without previous heating. A very obvious objection to this method is that the xylol soon becomes so saturated with paraffin that it is no longer effective. As a matter of fact, the xylol begins to give inferior results before a dozen slides have been passed through it. After the paraffin had been removed, it was our custom to rinse the slide in absolute alcohol and then successively in 95 per cent., 85 per cent., 70 per cent., 50 per cent., 35 per cent., until an alcohol was reached which had about the same strength as the stain to be used. This method gave good results, but, we believe, is unnecessarily tedious when dealing with thin sections in which nearly every cell is cut open on one side or another.

The method which we are employing at present is by no means new. It is, rather, a modification of a method in vogue as long as fifteen years ago. This method, as we are using it at present, is as follows:

The slide is heated gently—preferably on the paraffin bath—until the paraffin melts. The slide is then immediately flooded with good turpentine, which instantly removes the paraffin. A teaspoonful or, at most, two teaspoonfuls of turpentine is sufficient, if applied immediately, before the paraffin has begun to cool. Even a smaller quantity is sufficient if judiciously applied. We recently put up a lot of 112 slides, nearly all with paraffin ribbons  $10\mu$  thick and 50 mm. long. We used only 100 c. c. of xylol and 200 c. c. of alcohol. The paraffin was completely removed. It is best to keep the turpentine in a bottle with a rather small neck, so as to facilitate the pouring. The slide should be held at an angle of about  $45^\circ$  in order that the paraffin may be carried off at once. We do not use the turpentine a second time, but drain it into a waste jar. Since the first two or three cubic centimeters, if applied before the paraffin has begun to cool, will remove almost all of the paraffin, the slide may then be flooded several times and this turpentine may be poured back from the slide into the bottle. Xylol

may be used in the same way, but is a little more expensive and does not seem to give any better results.

The turpentine is now rinsed off with 95 per cent. alcohol—absolute alcohol not seeming to offer any advantages. The alcohol is kept in a bottle with a narrow neck and is poured upon the slide, just as was the turpentine. Two teaspoonfuls, judiciously applied, are usually enough to remove the turpentine. We do not use this alcohol a second time. The slide is now placed directly in any stain, whether alcoholic, anilin or aqueous, without passing through any of the various grades of alcohol. The only alcohols which we now keep in Stender dishes are 50 per cent., 95 per cent., and absolute. The 95 per cent. is retained merely to avoid an unnecessary weakening of the more expensive absolute alcohol, while the 50 per cent. is used to wash out various stains, like safranin, etc.

With thick sections this method is not so satisfactory, since plasmolysis may result even in fixed material. We still use a closely graded series of alcohols for hardening and dehydrating material preliminary to embedding in paraffin.

University of Chicago.

CHARLES J. CHAMBERLAIN.

### An Electric Thermostat.

Last fall the writer had occasion to use a paraffin bath, but had no gas in the laboratory. This led to the construction of an electric heater. After some experimentation, we constructed hastily and rather roughly a thermo-regulator,

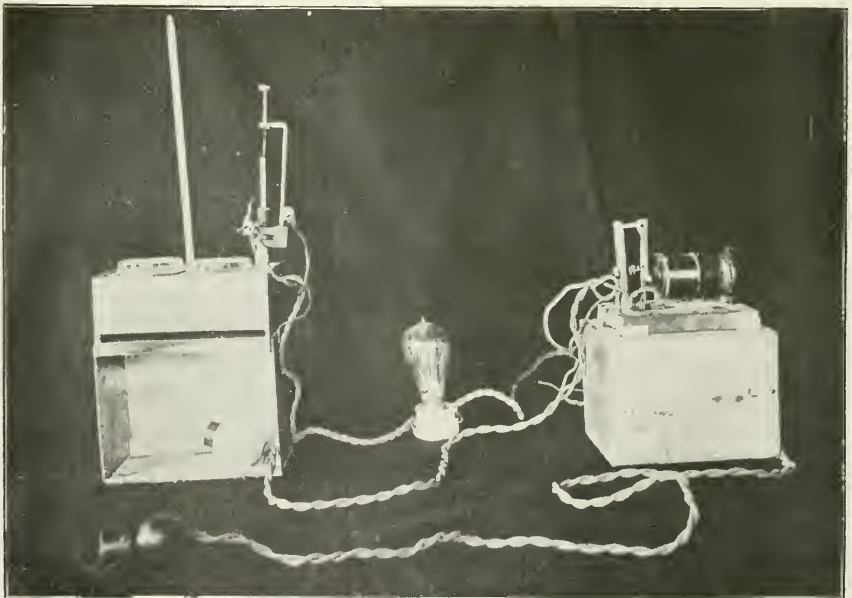


FIG. 1.—Photograph showing the thermostat as used in heating a paraffin bath.

that we have used and found to be fairly sensitive. We were able to keep the temperature from varying more than  $\frac{1}{4}$  of a degree C. By making the mercury cistern larger, there seems to be no reason why the variation may not be reduced.

To a common electric light plug (A) was attached several feet of electric light cord. Some distance from the plug, the insulation was removed from the two cords and two other cords were soldered to them. This provided us with two circuits from the same plug. The first circuit carries the current for the lamp (B). This 32 C. P. lamp we found to be more than sufficient to heat our little bath. The lamp is controlled by the make and break (C) which can be made to break the circuit by a slight movement to the right. The other circuit from the plug (A) passes through the magnet (E) and the thermostat (H). The two electrodes of this circuit are represented by the column of mercury in the glass tube (d) and the *soft iron wire* (e) projecting down into the tube.

When the current is first turned on, the lamp (B) which is placed beneath the bath to be heated raises the temperature of the bath. The lower part of the

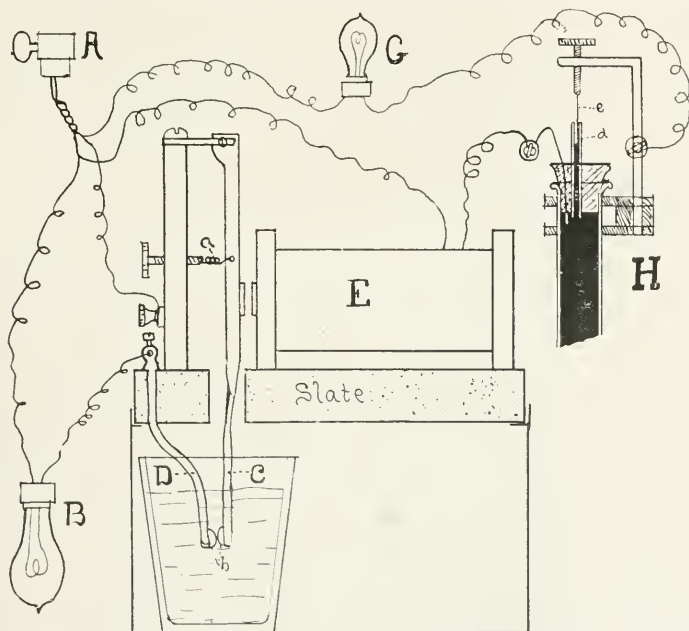


FIG. 2.—Diagram to show thermostat as connected for heating. A., electric light plug inserted in socket; B., electric light used for heating bath; C., make-and-break; D., post; E., electro magnet; G., lamp used as a resistance; H., thermostat; a., spring; b., silver contacts; d., glass tube containing mercury column; e., soft iron wire.

thermostat (H) rests in the bath. As the temperature rises, the mercury also rises in the glass tube and comes in contact with the iron wire closing the circuit through the magnet (E) which, pulling against the spring (a), overcomes its tension and moves the make and break (C) away from the post (D), thus breaking the heating current. As soon as the temperature falls enough to break the circuit through the thermostat, the make and break returns to its former position and the lamp (B) once more starts to heat the bath.

Fearing that an arc would be formed and that even rather heavy contacts might be rapidly fused, we immersed the contacts (b) in a cup of kerosene. This arrangement proved quite satisfactory. The oil acting as an insulator

greatly reduces the arc. Rather heavy silver contacts were found to be efficient. To avoid any danger of fire, we placed the cup of oil in a tin box which also served as a stand for the slate base.

The thermostat may need a more careful description. Two holes were made through a rubber cork. Through the one in the middle a glass with a small bore was inserted. A copper wire was placed through the other opening near the side of the cork. A test tube was filled with mercury and the cork was inserted. Enough mercury was poured into the glass tube to raise the level of the liquid a little way above the cork. The test tube was then fastened to a frame made of insulating material. A copper post firmly fastened to this frame projected some distance above the top of the glass tube. Turning at right angles it held a burr at its free end. A brass thumb screw to the end of which was soldered a soft iron wire screwed up or down through the burr.

To regulate the thermostat all that is necessary is to raise the end of the iron wire high above the mercury. When the bath has reached the desired temperature, screw the wire down until it just touches the mercury. The lamp (G) was placed in the circuit as a resistance to keep the magnet (E) from becoming overheated. In selecting the wire for the thermostat two properties were taken into consideration. The wire must not readily amalgamate with the mercury or it will be eaten away, thus destroying its accuracy. If the metal has a strong adhesion for mercury the top of the mercury column will be drawn out into quite a long neck before it breaks the circuit. A weakness of this sort greatly affects the variation of the temperature. We have not experimented very extensively, but we find platinum to be very poorly adapted on account of adhesion. Soft iron appears to work very nicely. The thermostat presents one especial weakness. When the circuit is broken a spark is formed which gradually uses up the iron wire. When immersed in light oil this difficulty is obviated, but each spark carbonizes a little oil. After using for several weeks, the tube is blackened to necessitate the removal of the wire and the cleansing of the tube. If some one can find an oil that will not readily carbonize, it will certainly add greatly to the value of the thermostat.

I see no reason why this device cannot be adapted to larger baths, incubators, and ovens. All that would be necessary would be to substitute a heating coil for the electric lamp. If a constant temperature with less variation is needed it can be obtained in either of two ways. Two heating coils or lamps can be used; the one which should be insufficient to heat the bath in operation all the time, the other regulated by the apparatus described merely keeping the temperature up to the proper point. Enlarging the mercury cistern of the thermostat will also reduce the variation.

JESSE J. MYERS.

Michigan Agricultural College.



## Sectional Specimen Cabinet.

The microscopical work conducted by the Bureau of Chemistry is such that a large and ever increasing number of standard authentic samples as well as common commercial products is required as reference material. The work performed at present includes such subjects as various food materials, spices, starches, flours, coffees, teas and cocoas, and textile goods, paper, etc. In order to meet the requirements of such a collection, a sectional specimen cabinet was devised, such that new units can be added from time to time without interfering with the general appearance of the cabinet. It embraces certain features of the cabinet described in the JOURNAL last year.\*



FIG. 1.

At present the cabinet consists of four units, a base and a cap-piece, and occupies a floor space of 35 by 20½ inches and stands 56 inches high (Fig. 1). The top section contains the card catalogue. The bottom section is fitted for storing microscopic slides, while the other two are arranged for storing reference samples in small glass jars. A detailed description of the separate sections will make clear the construction of the various units.

\* A Convenient and Economical Cabinet for Microscopical Slides, JOURNAL OF APPLIED MICROSCOPY, April, 1902. U. O. COX.

The card catalogue unit is  $9\frac{3}{4}$  inches high over all and holds two drawers, each  $30\frac{1}{2}$  inches long by 17 inches wide and 4 inches deep inside. Each of these is divided by partitions from front to back into 5 apartments capable of storing catalogue cards  $3 \times 5$  inches in size (Fig. 2 *a*).

Each of the units for reference samples is  $12\frac{1}{4}$  inches high and holds 7 drawers  $30\frac{1}{4}$  inches long by 17 inches wide and  $1\frac{3}{16}$  inches deep inside. By thin partitions each drawer is divided into five rows of 21 apartments each (Fig. 2 *b*). The samples are placed in one ounce screw cap jars and when placed in the drawer the labels are in plain view, which is much more convenient than where the jars stand upright.

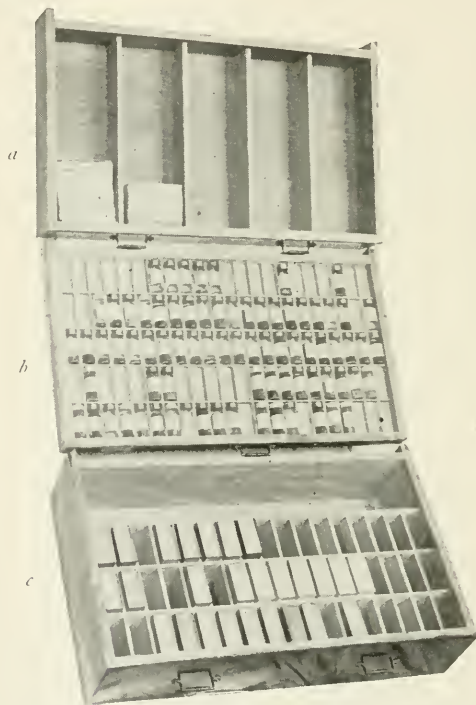


FIG. 2.

The unit for storing slides is 16 inches high and carries two drawers, each  $30\frac{1}{2}$  inches long by 17 inches wide and 7 inches deep inside. Partitions divide each drawer into three rows of 17 apartments each. Each apartment is  $3\frac{7}{8} \times 1\frac{9}{16}$  inches, being thus large enough to receive the conventional slide box holding 25 slides (Fig. 2 *c*). The rear  $4\frac{3}{4}$  inches is left vacant so as to hold the drawer from dropping down when boxes in the back row are being handled.

We have found the cabinet very convenient in form and it is at present capable of holding 12,000 index cards, 1470 reference specimens and 2550 microscopical slides. By numbering the slide boxes and apartments of the drawers, and finally cataloguing the collection, any slide can be located at a moment's notice.

BURTON J. HOWARD.

## Worcester's Formol-Sublimate Fixing Fluids.

The killing and fixing fluids here described have been in use in the Zoölogical Laboratory of the University of Michigan for a number of years. They have proven so satisfactory for certain purposes that it has seemed desirable to give other workers the benefit of the formulæ. The fluids were discovered and first used by Prof. Dean C. Worcester during the time when he was a member of the staff of this laboratory. He used them with very satisfactory results in the investigation on which he was then engaged, and had intended to publish the formulæ along with the general results of the investigation. For various reasons it has not been possible to publish the results of this work, and, with the approval of Professor Reighard, the formulæ of two of the most important and useful of Professor Worcester's killing fluids are here published.

The fluids are essentially combinations of formalin and corrosive sublimate made in the following manner:

### Formula I—Formol Sublimate.

To 10 per cent. formalin add sufficient corrosive sublimate to make a saturated solution.

### Formula II—Formol-Sublimate-Acetic :

Formol-Sublimate fluid (Formula I),	-	-	-	-	9 parts
Glacial Acetic acid,	-	-	-	-	1 part

Formula I is especially adapted to the killing and fixing of Protozoa. For this purpose it is extremely satisfactory. Some years ago the writer made a rather extensive series of tests to discover the best fixing agent for infusoria. Nearly all the fluids which had ever been advocated for this purpose were tried, and by far the best results were obtained with this formol sublimate combination of Worcester. The fluid fixes cytological details with great precision and completeness, and at the same time it kills so quickly that it is possible to fix such delicate infusoria as *Paramecium* without producing any appreciable distortion of the body as a whole. The importance of this will be appreciated by anyone who may have tried to fix Protozoa without distortion by any of the ordinary methods.

Formula II is especially adapted to the fixation of teleost eggs, and of embryological material in general. I have used it on amphibian eggs, and have found that it does not produce coagulation and cloudiness in the gelatinous envelopes if thoroughly washed out.

Material should be fixed in these fluids about as with an aqueous solution (saturated) of corrosive sublimate. Washing may be done in water or in 4 per cent. formalin. Four per cent. formalin may be used for preservation, or the material may be carried up the grade alcohols and preserved 70 per cent.

It is believed that these fluids deserve to, and will, become standard cytological reagents.

RAYMOND PEARL.

## New Method of Fastening Celloidin-Embedded Objects to the Block.

After following for several years what were to me the most commendable of the various details set forth in books and journals in reference to celloidin-embedding and the relation of celloidin-embedded objects to blocks, I have devised a plan of fastening such objects to the block which very much simplifies their handling. I have found this method very successful, and, since testing its efficiency, I have again searched the literature at my command but fail to find that the method has been described, so I report it as new.

The method is very simple. Remove the celloidin-embedded tissue from the alcohol in which it has been preserved and dry it by blotting with filter paper and completing the drying by letting it lie upon the table with the side which is to be next to the block upward. The drying does not need to be so thorough as to cause the slightest shrinkage or distortion of the object. Then take up the section between the thumb and forefinger or with forceps and dip the side which is to be applied to the block into ether contained in a small Stender dish and keep it in contact with the ether for 10 to 20 seconds. This will soften the celloidin. Then transfer it quickly to a dry block and press down upon it for one-half to one minute. It is now ready to be clamped into the holder, and by the time it is adjusted to the knife it will be so firmly adherent that it will not spring or be loosened by cutting. When enough sections have been cut, remove the tissue from the block and put it into the alcohol. Only 40 to 80 seconds will be consumed in fastening the tissue to the block exclusive of the drying, which must be done in any event. The whole process need not take more than 2 to 3 minutes.

In order to follow this plan it is necessary to embed by putting the pieces of tissue into thick celloidin in a dish, such as a Stender dish, with the side from which you wish to cut downward, and letting the celloidin slowly harden around it. When sufficiently hard, cut the embedded tissue out and put it into 80 per cent. alcohol to preserve until it is wanted for cutting, when it is put upon the block as above described. The advantages of this method are as follows:

1. Only one block for each microtome in use is needed. Thus the problem of blocks is almost entirely done away with.

2. It greatly simplifies the storage of the embedded objects.

3. It necessitates the embedding in dishes, which is always the best.

Experiment Station, Ames, Iowa.

JOHN J. REPP, V. M. D.



## Rapid Method for Examining Bacteria in Tissues and their Staining with Haematoxylin.

It is hard to find any other method or stain that has not already been modified in many ways. Take for example the freezing or rapid preparation of tissues by the Cullen method, to which a fresh impetus has been given. The primary method, I believe, was originated many years ago either by Rutherford or by Prof. E. Klein. Any way, diagnoses were made in the operating and autopsy rooms when these teachers were beginning the founding of histologic and pathologic laboratory work in England and Germany, following their masters such as Billroth, Kölliker, Frey, etc. In this country in '87 such work was done, specimens being cut, stained, mounted and diagnosed in 10 to 25 minutes in the University of Michigan, and in Chicago in '92 or possibly before. The introduction of formol was some gain, but did not possess material advantage over  $\text{OsO}_4$ , except expense perhaps.

The following method may be of use in some work, and as it is not much known this fact must be my excuse for giving it. Originality is not claimed, for at this date I am not able to place the laboratory from which I got it.

- (a) Cut organs into small cubes as soon as they can be had.
- (b) Write on pieces of filter paper (*fine grain*) the points of interest, name, etc. Place the tissue on the paper with the items at the side. Place in alcohol.
- (c) Harden during a period ranging from a few hours to a few days, changing the alcohol frequently.

Now fasten the tissue to the block with the following mixture:

Glycerine	-	-	-	-	-	-	-	4 c. c.
Gelatine	-	-	-	-	-	-	-	1 gram
Water	-	-	-	-	-	-	-	2 c. c.

Heat and dissolve to a thick mass, when the tissue will be found to be fastened firmly. Place in alcohol, preparation side down, for 12 to 24 hours. Cut.

This applies to organs like the liver, kidney, and the more solid structures. Formol can be used to harden if the pieces are very small, as it lacks deep penetrating powers. Formol gelatine forms an insoluble compound, but clear, and is much used for eye museum preparations.

In connection with this I would like to ask the question if any one has tested the power of hæmatoxylin as a bacillus or bacterial stain. In making a rapid examination of what was called an ulcer of the nose, I found my hæmatoxylin sections showed many well stained bacteria, both on the periphery and interiorly. The Löffler's blue and carbol fuchsin did not show them nearly so well nor in so many numbers.

I find one of the quick methods is Kischensky's, as follows:

- (a) Place a drop of weak solution of carbo-fuchsin on the cover, mix with a very small quantity of the pure culture to be examined and spread thinly and evenly.
- (b) Heat in the fingers gently over the flame.

(c) In pus, blood, etc., you can mix the above stain with an alcoholic methylen blue solution.

The *gain* is in quickness, simplicity, cleanliness and excellent results in staining, the *Flagellæ* being seen while the field is kept clean and unstained.

Rogers Park, Chicago.

V. A. LATHAM.

## A New Sling Psychrometer.

A couple of years ago my attention was called to the desirability of having something a little better than the ordinary sling psychrometer for use in my classes in botany. As is well known, it requires a good deal of free space to enable one to manage the ordinary sling psychrometer without striking some object. Those of the ordinary pattern which we have used in the field and also in the laboratory are invariably broken after a short time. The suggestion was made that the whirling of the thermometers might be accomplished by some

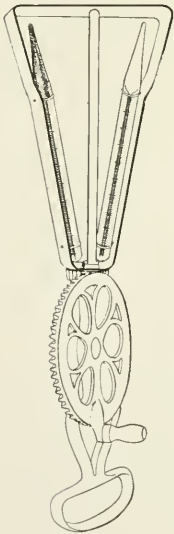


FIG. 1.

mechanical means, and after a number of trials, and the manufacture of several instruments, the one here shown in the figure was made. It consists of a handle, shown at the bottom (Fig. 1), and to this is attached a bevel cog-wheel which works in another smaller wheel, turning the upper portion of the apparatus. To this upper portion are attached the two thermometers, the one having its bulb covered with cloth and the other free. By wetting the cloth on the one thermometer and then rapidly turning the crank attached to the wheel, a very rapid motion is given to the frame which carries the two thermometers. In many trials made with this apparatus, it has been found that the effect of this rapid rotation is exactly the same as that produced by the slinging motion of the ordinary sling psychrometer. The advantage of this little apparatus is that it can be used without danger of breaking the thermometers. This form is of still greater advantage in work in the field, especially in forests and thickets. It is not at all difficult to obtain the relative moisture of the air in the middle of a thicket by this instrument, whereas it would be utterly impossible to do so by

the use of the ordinary sling psychrometer.

In making the instrument thus far I have found that the cheapest plan is to purchase a "Lyon Egg Beater" and to have a mechanic remodel the upper portion in the way indicated. I have a number of such instruments which cost me a little less than two dollars apiece, and they are much more serviceable than my sling psychrometers that cost twice as much. No doubt a skillful mechanic can make a neater looking instrument than these made out of transformed egg-beaters, but it will probably not do any better work.

University of Nebraska.

CHARLES E. BESSEY.

## The Museum.

### IX.

#### THE CASE.

It seems possible that a few simple forms in cases with a reasonable flexibility in dimensions will meet all the requirements of the museum curator. Considerable ingenuity can be expended in devising cases of varying forms, but it hardly strengthens the impressive effectiveness of a museum equipment to fill halls with an allotment of cabinet inventions. If the purposes of a case be analyzed they will be found to embrace, primarily, room for an object to be so exhibited that it can be seen from the outside of the case *to the best advantage*; secondarily, security against theft and dust; and thirdly a requisite workmanship and material, to make it a pleasing visual object. As to the point of dimensions cases must be high or low, deep or shallow, small or large; as to location they are wall cases or floor cases. In these aspects you cannot introduce further variation except by *suspension*, an exigence rarely contemplated. As to shape the element of diversity may be considerably extended and generally with unfortunate results. While, under exceptional circumstances, as in art museums, or gem exhibits, round, polygonal, elliptical, sand glass, trimmed and compo-

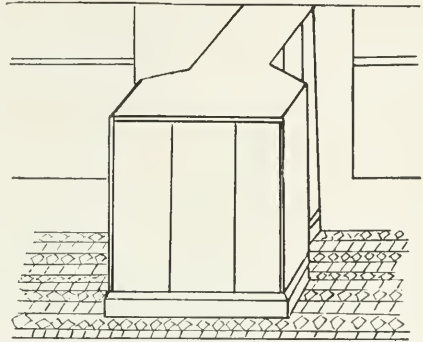


FIG. 37.—T-case showing front on hall and alcove.

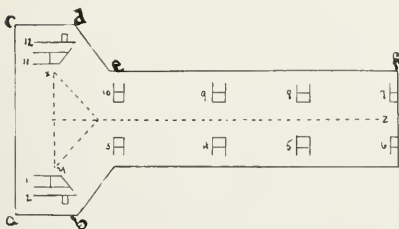
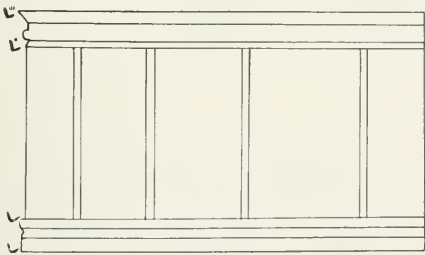


FIG. 38.—T-shaped pin case.

site cases may be introduced in the equipment of special rooms, or for the more æsthetic presentation of rare and decorative objects, usually it will be found satisfactory to confine all forms practically to the parallelopipedon or *box*, whether flat, produced, inclined, or cubical, and to have the glass fronts plane.

A case which might seem to offer a wide departure from the above stringent recommendation, and yet a case which has always appealed to the writer, in spite of very influential strictures against it, is a case used in some halls of the New York museum, though probably in future to be abandoned.

It is a peculiar and admirable form of pier or alcove case developed by Mr.

Calvert Vaux in the first section of the American Museum of Natural History. It is a T-shaped case—in reality two parallelopipedons connected by a slope—designed to form a front upon three sides, two aspects against the alcove itself, and the third upon the hall face of the case (Fig. 37).

In this disposition, of course, the alcoves are lighted by a window, which should be almost the entire width of the alcove itself. By removing the shelving, large enough compartments are formed for single large figures, as mammals, while their generally good illumination, increased also by the inclined ends, their great capacity, their structural interest, and the room-like effect of the alcoves produced by their approximating distal ends, all combine to attract for them a more considerate inspection than they have received. The dimensions of this example of pier case are as follows—compare (Fig. 38): plan, a-b, 3 ft.; a-c, 9 ft.; d-e, 4 ft.; e-f, 12 ft.; elevation, L-L', 1 ft.; L'-L'', 7 ft.; L''-L''', 1 ft.; x, y, 3



FIG. 39.—Old wood hall with Jesup Hall of woods in New York Museum.

is the intersection of a vertical partition with the base of the case, forming a background and reflecting surface for the objects in front, and dissipating the confusion of cross lights, as well, and more particularly limiting the vision to the shelves immediately in front of the visitor; 1, 2, 3, 4, 5, etc., are the bare sockets of uprights armored with ratchet strips for the attachment of brackets to support shelving.

These are strong and elaborate cases, requiring considerable labor for their construction. The construction itself is fundamentally determined by two T-shaped wooden forms. The form at the bottom has an iron sheath at the base, and an iron hoof forming a bearing at the base of the doors, and projecting so that standing on it, or any accident, will not splinter it, as if it were wood. The form at the top has a slight and perhaps quite unnecessary iron sheath. Upon the two forms respectively, floor and roof are laid, while the partitions of the sash spaces, which are iron, support the latter. The whole is practically wood



with a slight interjection of iron; the mouldings are simple, the wood black walnut oiled, the angles in front rounded, the depth of the frame (also iron) holding the glasses (lights) two inches; the doors are thus of single panes of glass swung on iron pins in sockets, and locked by the bolt or Ienck's lock.

Along the main hallway the extended ends of these cases form a broad façade-like effect, quite superior to the narrow ends of the ordinary pier cases (Fig. 39).

A peculiar form of T-shaped case has been adopted by President Jesup for his wood collection in the New York Museum. They are the T-shaped cases just described, but in different proportions, and in one instance (Fig. 40 A) very greatly modified. Although these are magnificent cases, admiration previously expressed for them (*The Making of a Museum*) must now be practically suppressed or withdrawn. They are ungainly and ill conceived; the combination of the strict T-shape with a bulging proximal base is inartistic (A) and the elongation (B) of the shorter type of T-case also disappointing.<sup>1</sup> They were, however, designed for a special purpose, which they seem to meet, and their finish and appearance is undoubtedly striking. Their bases are completed with a five-inch strip, one-half inch thick, of white marble, protecting them against defacement.

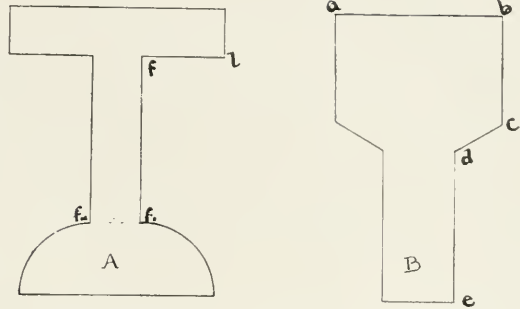


FIG. 40.—Case forms in Jesup wood collection.

This brief allusion to an interesting experiment in cases, which the writer believes is not generally endorsed, may be considered episodic. The discussion is now properly resumed of Wall and Floor cases.

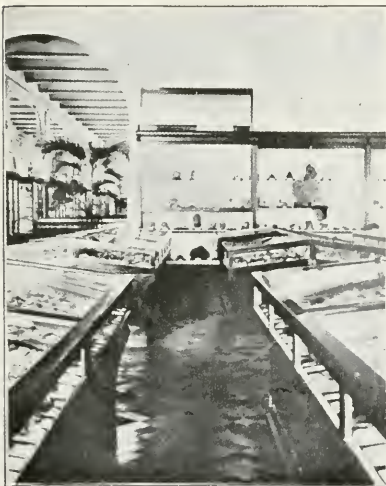


FIG. 41.—Showing sash half open of doors pushing upward.

#### WALL CASES.

Wall cases proper are those built up against walls extending considerable distances, in fact, covering all the wall surface of a room or hall as high as the case itself reaches. Such cases are variable in depth according to uses. Wall cases are frequently poorly lighted when placed between windows, and when they are themselves shallow. Their best position is between windows or opposite windows—or both. Such positions in long museum halls and along interior

<sup>1</sup> The measurements of these large wood cases are as follows (compare figures): a-b, 9 ft.; b-c, 6 ft.; c-d, 4 ft.; d-e, 12 ft.; h-i, 25 ft.; i-l, 4 ft.; f-f', 10½ ft.; f'-f'', 4 ft.

walls where the requirements of construction are such as to forbid windows or skylights are of course impossible. Recourse must then be had to pier wall cases extending well out into the hall, or the use of the wall along such exposures as are insufficiently lighted must be minimized, or every device adopted for getting the best diffused illumination possible. A very obvious remedy, where on one side of a hall a long wall space is available and which cannot be pierced with windows, while the opposite wall may form the front of the building, is to open the latter with high or broad windows, so as to flood the closed wall with light, while at the same time abundant diffused light is secured for lighting the central exhibits of the hall. In such a case the central exhibits should be in flat table cases in order that the least obstruction should be offered to the incident light.

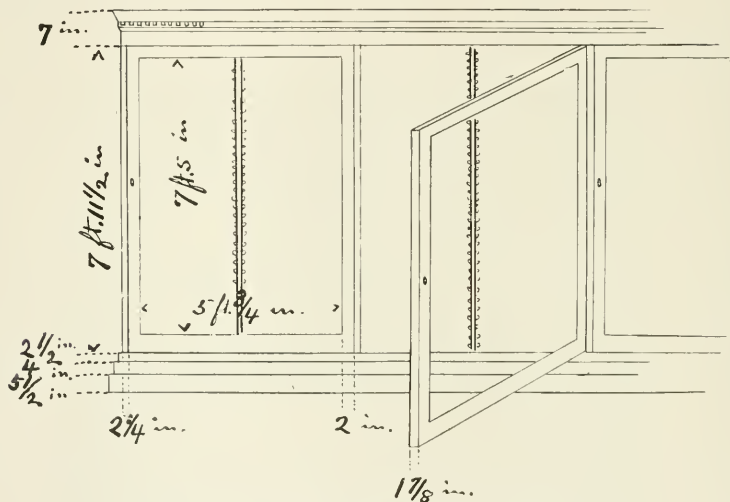


FIG. 42.—Typical Wall Case with dimensions.

The practice of using glass tops to cases, as recommended by Dr. Goode, seems questionable, except in group cases where the appearance of the case itself is improved. Dust soon accumulates on the glass or it becomes otherwise dirty, and it requires frequent cleaning, while when objects are raised above the cases the glass is an obstruction and danger to their manipulation. Dimensions of wall cases of course vary, their depth being dependent on the character and size of the objects they are intended to hold. A depth of three feet seems widely serviceable.

The doors of all upright cases should be swing doors, opening outward for the one sufficient reason that they can be easily cleaned. Dr. Goode's advocacy of doors pushing upward (Fig. 41) was induced by supposed value of the broad glass pane such an adjustment allows, which of course may have advantages. But in practice it will soon be found that the lid which is pushed open by the ascending sash and which falls again upon the latter's descent cannot be kept dust proof, and that every time it is opened it dispenses some of its accumulations upon the objects and shelves in the interior of the case. It is altogether

a bad arrangement. The difficulty of cleaning the whole door properly seems to outweigh all advantages.

The height of wall cases has been much debated, those of the New York museum having been censured on account of their considerable height. In that case the objection does not seem to be well taken. There should be some relation of congruity between the height of a hall and the height of the wall case in a museum. If the former is lowered the latter can be also, but a very high ceiling with too low wall cases and immense unused wall spaces is distinctly disagreeable. If, however, the wall spaces are used, or if large objects surmount the cases, reaching up into the unoccupied and useless vacancies of the wall, then the wall cases may be reduced in height. The height of the ceilings in the American Museum of Natural History, in the larger halls, is about 22 feet, and that of the wall cases is from 9 to 10 feet, outside measurement, or more than one-third the height of the ceiling. This brings the interior top shelf of the cases above the line of vision of most men by about one and a half feet, so that tall and coarse or less significant specimens are restricted to it. This seem no particular disadvantage and results in no educational loss. It is also possible to lower such a top shelf and leave the upper wall of the case empty or distinguish it by some planular exhibit, maps, drawings, explanations, etc., etc.

Effectiveness of linear relations counts for something in a hall, and while the limit of 9 feet may be insisted on in high halls, a minimum of seven should be made imperative. The high wall cases contain more room, and especially permit an attractive spacing of the shelves. A deep moulding around the ceiling of halls, and the use of two colors on the walls of a hall, will also mitigate the altitude of high ceilings.

An example of a satisfactory wall case with broad sashes and good depth, chaste finish, and substantial construction is here given (Figs. 42 and 43) with dimensions. These cases are 7 feet 10 inches from floor to ceiling, inside measurement, have an *inside* depth of twenty inches, with each sash space provided at the back, opposite and midway in the sash spaces, with up-rights carrying ratchets for insertion of brackets (see below). The door closure is indicated in the section on Fig. 44, the back rim of the door falling behind a, and fitting tightly against b; the doors swing on pins, top and bottom, perforating the metal plate which is given in Fig. 44 c. A plate is bedded in the door, and in the case.

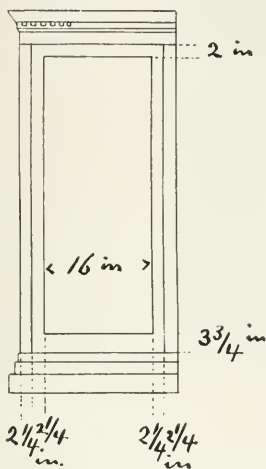


FIG. 43.—Depth and dimensions of side of typical wall case.

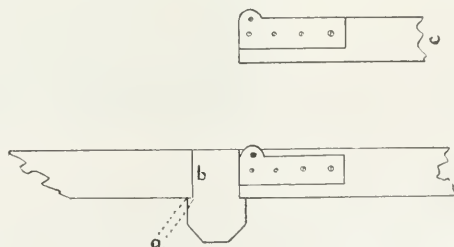


FIG. 44.—Plate hinge for door of typical wall case.

Figures 45 and 46 show two other wall cases with respectively broad and narrower doors; in the first instance the doors slide past each other on tracks.



FIG. 45.—Wall case furnished with doors running on tracks.

This latter is a very deep case for the reception of mammals, and has an inside measurement of nearly four feet.

In a few instances sliding doors running on tracks at the base and top of the case have been instituted, but while this affords a reasonably, though imper-



FIG. 46.—Wall case furnished with doors swinging outward.

fect, dust-proof case, the difficulty of cleaning the glass again intervenes, as the cleaner must enter the case, and all shelves and objects, within, be removed.

American Museum of Natural History.

L. P. GRATACAP.



## The Technique of Biological Projection and Anesthesia of Animals.

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### XVI. DIFFERENT TYPES OF CELLS IN WHICH LIVE ANIMALS AND PLANTS ARE MOUNTED.

In considering the question of cells in which to mount live organisms for projection, three conditions which are essential to the greatest success should be kept in mind. First, the live organisms will need sufficient water or air, according as they are aquatic or aerial, to sustain life during the experiment; second, the organism should be confined within reasonable limits relative to its size, especially as regards the depth of the cell in the line of the optical axis of the microscope, so that the specimen may be easily kept in focus; and, third, the cell should be easily adjustable and movable on the stage of the projection microscope.

The cells shown in the accompanying engraving, Fig. 9, include nearly all the available shapes of glass, or glass with metal or rubber, cells now offered in the American and European markets and, in addition, some designed and made by the author to meet special requirements. All of the cells have polished surfaces in the parts which are in front of or behind the mounted organism in order that the maximum amount of light and the best possible definition may be obtained in the projected picture on the screen. Cells with rough or ground glass bottom are not at all satisfactory in projection work.

In Fig. 9, the cells numbered 1 to 13, also 15, 21, and 22, have loose covers which are placed over the specimen after it is put into the cell; while 14 and 16 to 20 are open at the top for the reception of the specimen, the front and back being cemented on with water-tight joints.

The cell numbered 1 is a polished plate glass microscopical slide 3 in. by 1 in. for use in mounting amœbæ, bacteria, infusoria, and other microscopic organisms which are shown by the use of objectives of high power and correspondingly short working distance; 2 is a hollow-ground or culture slide, 3 x 1 in., with an elliptical concavity; 3 is similar to 2, but has a circular concavity (B. & L. Opt. Co. Cat. No. 1306); 4 is a culture slide having a slightly concave center which is surrounded by a deep groove; 5 has an elliptical concavity about one and one-fourth inches long, seven-sixteenths wide and one-eighth inch deep in the center; 6 has a similar but wider and shallower concavity in a plate of glass which is cemented on a glass slide; 7 is an embryo glass having a concavity one and an eighth inches in diameter in a plate about four by one and one-half inches; 8 is an ordinary embryo glass one and an eighth inches square cemented to a glass slide; 9 is an embryo glass with only the bottom and the concavity polished; 10 is a form of life box simpler than B. & L. Opt. Co.'s No. 1830; 11 is a glass cell about four inches square and three-eighths inch deep; 12 is a small size, one and three-fourths inches square, of the same form as 11, and other sizes are in the market; 13 is an embryo glass two inches square; 14 is a life cage

(B. & L. Opt. Co. Cat. No. 1834), with adjustable partition, wedge and spring ; 15 is a compressor (B. & L. Opt. Co. Cat. No. 1264), with the author's modifications for making it into cells of different depths and shapes ; 16 is a cell with polished plate glass faces two and a half inches high by one and three-eighths

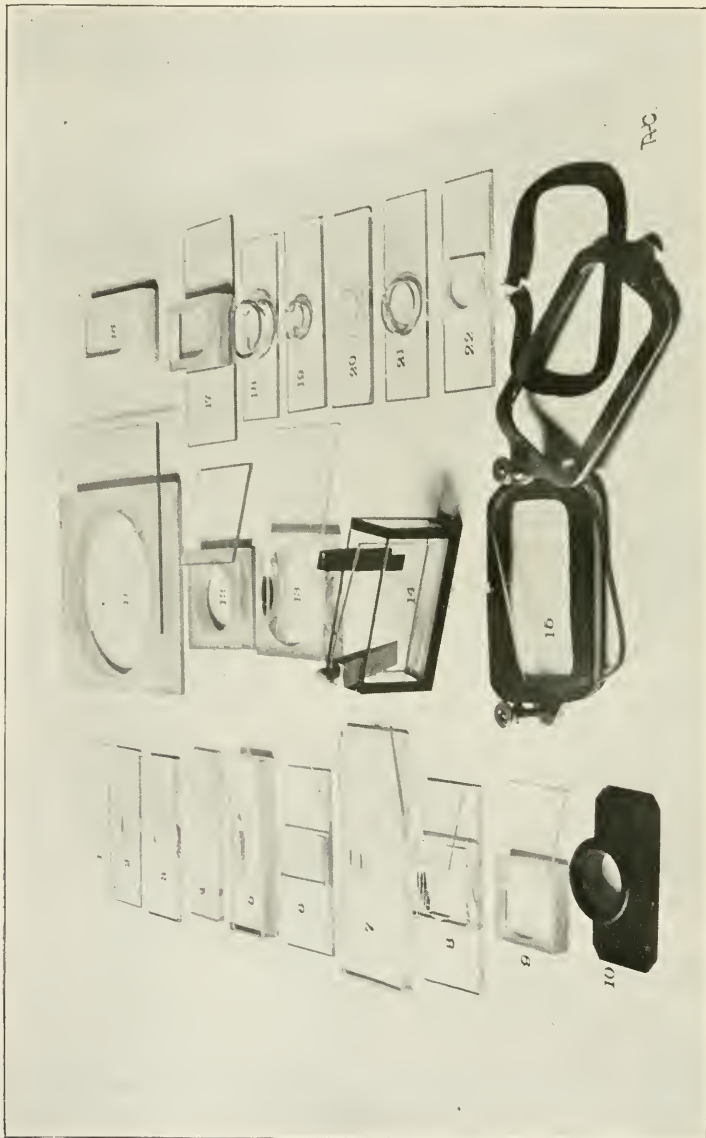


FIG. 9.

inches wide and open at the top ; 17 is similar to 16 but is lower, deeper from front to back, and is cemented to a slide ; 18 and 19 are open ring cells made by cutting out a section of a glass ring (B. & L. Opt. Co. Cat. No. 1250), cementing the open ring on a plate glass slide which forms the back of the cell while

the front is formed of a thick cover-glass cemented on the ring ; 20 is a cell made by cementing a thick cover-glass—square rather than a circle—over three-fourths of the hollow in an ordinary hollow-ground slide like 3 ; 21 is a closed ring cell made by cementing a glass ring on a plate glass slide ; and 22 is a better form of ring cell, because of the larger surface for attachment to the slide.

The most generally useful forms are numbers 1, 3, 6, 7 or 8, 10, 11, 13, 14, 15, 17, 18, and 22. Number 14 is improved by cementing it on a glass plate as in 17, and it may be used with many organisms for which 11, 13, and 17 are preferable.

For fish three or four inches long, crayfish, large tadpoles, and objects of similar size a large glass cell made like 16 with a length of about seven inches, height and thickness from front to back as great as the lantern will carry, is most desirable. Some dealers in projection apparatus sell these under the name of "alum tanks," those made entirely of glass well cemented being preferable to the kinds made of glass plates with metal and rubber fittings.

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A simple apparatus for the anærobic cultivation of bacteria, based upon the well known property of an alkaline solution of pyrogallic acid to absorb oxygen, consists of a battery-jar in which the cultures are placed on a suitable support. Over them is placed an inverted tin can or a sheet of tin foil, and over this some absorbent gauze and an inverted battery-jar. Water is poured into the outer jar so as to form a water-seal. A mixture of pyrogallic acid and caustic potash is introduced through a bent tube passing under the edge of the inner inverted jar and opening within the apparatus above the gauze, so that the latter is kept moistened by fresh absorbent solution falling upon it drop by drop. The outer end of the tube is connected with rubber tubing to a separatory funnel placed at a higher level than the jars. The solutions of pyrogallic acid (20 per cent.) and caustic potash (30 per cent.) are mixed in this funnel under a layer of paraffin oil, to prevent oxidation of the mixture. At first the gauze is stained dark brown ; when absorption is complete, a white spot appears where the absorbent solution falls upon the gauze. The rubber connection, with the separatory funnel, can then be broken, and the absorbent solution in the bent tube acts as a water-seal. The oxygen is removed from the jar in about thirty minutes, and an anærobic condition can be maintained for at least five days. The whiteness of the gauze is the guarantee that oxygen is excluded.—*Exchange*.

## Methods in Plant Physiology.

## XIV.

## HYDROTROPISM.

The response of plants to the presence of moisture is best demonstrated in the roots of young seedlings. In practice, however, the student usually finds some difficulty in furnishing an equable supply of moisture near enough the root to have its presence felt.

The illustration (Fig. 19) shows a method of performing this experiment by which good results may be obtained. The wire hoop by which the cone of netting is suspended is about 5 cm. less in diameter than a cylindrical battery-jar in



FIG. 19.—Hydrotropic response of roots.

which it is suspended. When the cone is nearly filled with damp sawdust, plant seeds of radish (*Raphanus sativus*) or white mustard (*Sinapis alba*) around the outside, a distance from the netting equal to three or four times the diameter of the seeds. While the experiment continues the cone of sawdust should be supplied with water once each twenty-four hours; after watering it, allow the water to drain away before replacing it in the battery-jar. No water should be allowed to stand in the jar nor should a cover be placed on the jar. The preparation



must be kept in the dark-room. Begin to make observations the third day after planting. If properly managed, the roots of the seedlings will be deflected from the vertical and follow the surface of the moist cone as they grow downward.

Another method of demonstrating the same response to moisture employs a moist cylinder, around which the seedlings are suspended. Cover a tall glass cylinder, e. g., a Welsbach lamp chimney, with moist filter paper and stand it erect in a battery-jar in which there is a shallow layer of water. Upon the top of the cylinder lay a disk of thin wood or sheet cork having a row of holes around

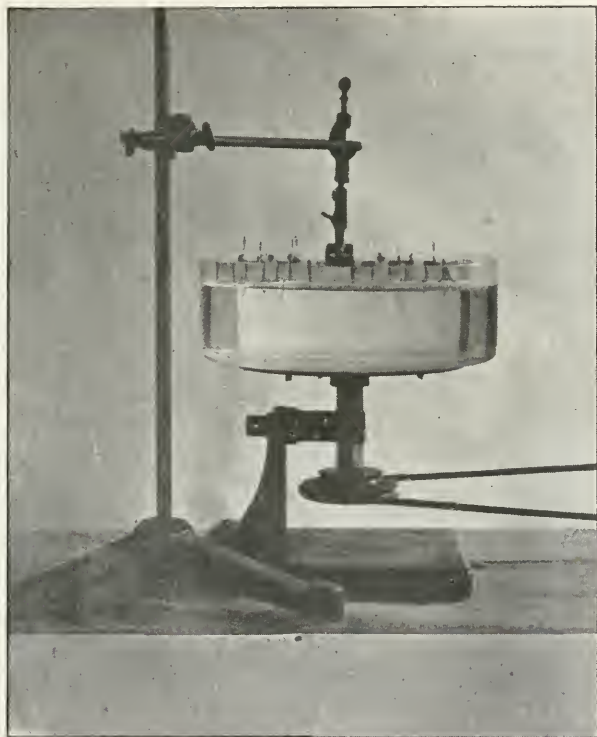


FIG. 20.—A centrifuge fitted for a demonstration of the rheotropic behavior of roots.

the circumference about 5 or 6 mm. from the outer surface of the cylinder. Insert in the holes seedlings of white mustard or radish, arranging them parallel to the surface of the cylinder. If the proper relations of moisture are maintained, the results will be the same as indicated above.

#### RHEOTROPISM.

When exposed to the force of a current of water, the roots of many seedlings give a very definite response by curvatures.<sup>1</sup> The most convenient way of conducting the experiment is to allow the roots to dip in a revolving dish of water. Figure 20 illustrates the method of revolving the dish on the horizontal plate of

<sup>1</sup> Cf. Newcombe, F. C. The Rheotropism of Roots. *Bot. Gaz.* 33: 177, 1902.

a klinostat. After the first few revolutions the water in the dish has practically the same rate of motion as the dish itself. The speed of the klinostat should be so adjusted that the water in the margin of the dish will have a velocity of 50 to 100 cm. per minute. Suspend seedlings of *Fagopyrum esculentum*, *Raphanus sativus*, or *Zea Mais* (popcorn) from the wooden bar in the clamp. Make observations at the end of fifteen to twenty-four hours.

#### TRAUMATROPISM.

The curves produced by wounds of various kinds are among the most pronounced and characteristic we know. (Fig. 21.) Select seedlings of *Lupinus albus*, *Pisum sativum*, or *Vicia Faba* when 3 to 6 cm. long and attach them to a

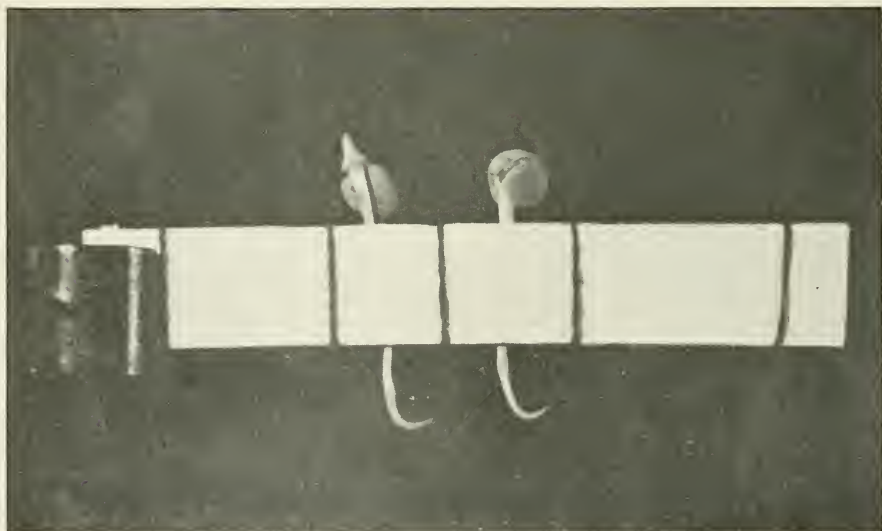


FIG. 21.—*Pisum sativum* seedlings ten hours after wounding with a hot rod.

suitable support in a damp chamber. Attach a bit of thin copper 2 mm. square to the sloping side of each root-tip. Fasten similar bits of copper or paper to other roots 3 or 4 mm. back from the tip. The thin pieces of copper may be obtained by hammering out the end of a copper wire. Other roots may be touched in corresponding places with a hot glass rod or a bit of solid potassium hydroxid. Care must always be taken against making a wound so severe that it kills the meristematic tissue.

Make observations ten to twenty-four hours after applying the stimulus and attempt to correlate the results.

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## A Review of the Methods of Staining Blood.

## XI—Concluded.

10. *For Staining Malarial Parasites.*

*Celli* and *Guarnieri* (1889) stained the living parasites in a solution of methylen blue in serous fluid. Ascitic fluid is collected with aseptic precautions in sterile test tubes. To these a sufficient quantity of methylen blue is added, which, after floating for a short time, sinks slowly to the bottom, coloring the fluid a deep blue. After filtering into another test tube the solution remains good for a long time. The finger of the patient is cleansed and punctured with a needle, and with a glass rod a drop of the staining fluid is placed upon the drop of blood that appears. From this mixture a drop is transferred to a cover-glass and allowed to spread out on a glass slide, a little pressure being used to spread out the red corpuscles and prevent the formation of rolls. The staining requires some time, and best results are obtained by leaving the preparation 1 to 2 hours in a moist chamber. The preparations are not wholly permanent.

*Feletti* (1890) stained the living parasites by the following method: A small drop of an alcoholic solution of methylen blue—1 part to 5—is placed upon a slide and allowed to dry by passing the glass over a flame. A drop of blood collected upon a cover-glass is placed upon the stained area and the cover-glass surrounded with paraffin. The methylen blue is redissolved in the blood serum and stains the parasites.

*Mannaberg* (1893) describes the following method of staining malarial blood with hæmatoxylin: The dry preparation is first floated for 5 minutes upon distilled water, then dried between blotting paper, then drawn several times through a very dilute solution of acetic acid (1 drop of acetic acid to 20 c. c. of distilled water) until it has completely given up its hemoglobin. The almost completely colorless preparation is then placed for 2 hours upon a fixing solution of picric and acetic acid (II, B, 2, 1), from which it is put into absolute alcohol for another two hours. Thereafter follows the staining for from 12 to 24 hours in alum hæmatoxylin (III, A, 3, a). Lastly clear up the preparation by means of acid alcohol (75 per cent. alcohol with 0.25 per cent. hydrochloric acid) and ammonia alcohol (3 drops of ammonia to 10 c. c. of 75 per cent. alcohol), wash in 80 per cent. alcohol and mount in xylo-Canada balsam. The preparation is first washed in water and acetic acid to remove the albuminous substance, which is apt to cause troublesome precipitates in the subsequent treatment with picric acid. Parasites and leucocytes stain blue, the red corpuscles remain colorless.

*Malachowsky* (1891) recommends Sahl's borax methylen blue (III, A, 4, c) after several minutes' fixation in absolute alcohol.

*Marchoux* (1897) recommends thionin (III, A, 9, a) for staining malarial blood.

*Schuffner* dries the preparations 6 to 30 hours in a place somewhat shielded from light, fixes in 5 per cent. glycerin and formalin 5 to 10 minutes, washes and stains in hæmatoxylin 1 to 10 minutes, according to strength.

*Chenzinsky* (III, C, 4, a), *Plehn* (III, C, 4, b), *Laveran* (III, C, 4, d), *Sforza* and *Mannaberg* (III, C, 4, h) have recommended double staining with eosin and methylen blue.

*Craig* recommends Fletcher's method for staining malarial blood: Fix the smears in 1 per cent. formalin in 90 per cent. alcohol  $\frac{1}{2}$  to 1 minute, stain with thionin (III, A, 9, c).

*Rees* recommends: (1) borax methylen blue (III, A, 4, d), (2) carbol-thionin (III, A, 9, b), (3) toluidin blue (III, A, 10), and (4) Delafield's hæmatoxylin or Mayer's hæmalum for staining malarial blood.

The neutral eosin-methylen blue stains (III, D, 5) are especially recommended for staining malarial blood. The polychrome dyes of this class double stain the parasites, the protoplasm and the chromatin staining differently.

For routine work in the diagnosis of malaria no staining is so simple and effective as Löffler's alkaline blue (III, A, 4, b) used after fixation with alcohol and ether (II, B, 2, b).

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ERNEST LINWOOD WALKER.

## Laboratory Outlines for the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

### THREE INTERESTING GREEN ALGAE.

XLI. *Oedogonium crispum* (Hass.) Wittr. (*E. nodosum* Keutz.) Family, Oedogoniaceæ.

This plant grows either upon or beneath the surface of ponds and pools, usually attached to various solid objects. It fruits most abundantly during May and June, and will grow well in aquaria.

1. Mount some of the filaments in water and examine under low power. Note that the filaments are unbranched and have a definite holdfast at the base. Draw.

2. Draw one of the cells under high power, showing the chloroplast with pyrenoids and the nucleus. Draw the basal cell (holdfast).

3. Nonsexual spore reproduction. If the filaments are in proper condition, any cell may develop into a zoospore and escape from the cell wall. Draw an empty cell. Draw a free-swimming zoospore. These have a circle of short flagella or cilia. Draw a zoospore which has settled down and enlarged and is developing a holdfast at the base.

4. Sexual reproduction. Note the oogonia, large cells each with an oosphere filled with food material. Draw. Find the opening at the base for the entrance of the spermatozoid. Draw an antheridium, usually consisting of two or three very short cells each of which gives rise to two spermatozooids.

5. Look for escaping spermatozooids and for spermatozooids which have entered the oogonium.

6. Draw an oogonium containing a ripe, thick-walled oospore.

7. When the oospore germinates it divides into four cells, each of which develops into a zoospore. The zoospores settle down and develop into new *Oedogonium* plants. An attempt should be made to have oospores germinate in





in a delicate sac and divides into a four-celled body (the sporophyte) which gives rise to four nonsexual zoospores. If material is at hand, study and draw.

8. Write out a careful description of the entire life history of this plant, noting especially that it has an alternation of generations.

XLIII. *Coleochaete pulvinata* A. Br. Family, Coleochaetaceæ.

Several species of *Coleochaete* are to be found growing attached to the surface of various submerged, fresh water plants. The species mentioned above forms hemispherical masses of closely packed, branched filaments. These masses are large enough to be seen with the naked eye and should be looked for on the petioles or lamina of water lilies and other hydrophytes.

1. Pick off some of the smaller and larger masses with a scalpel, mount, and examine under low power. Under high power draw a part of the branching filaments, showing the joint-like cells, each with a nucleus, chloroplast, and pyrenoid, and some with long, narrow, hair-like projections sheathed at the base.

2. Look for nonsexual reproduction by means of zoospores, a single one being produced in a cell. If these are present draw and find how they escape from the cell.

3. Draw the mature oogonium, showing the oosphere and long slender, open neck. How different from *Batrachospermum*?

4. Draw one of the antheridia, which are terminal or lateral flask-shaped cells of peculiar form easily distinguished from the vegetative cells. Each antheridium produces a single, biflagellate spermatozoid. Compare with *Batrachospermum*.

5. Draw a mature spermatozoid either free or in the antheridium.

6. Draw an oogonium in which the egg has been fertilized, and around which branches are developing from the base.

7. Draw an oogonium containing a ripe oospore and a cortical layer of close-fitting branches.

8. From material gathered in early spring or from prepared slides, study fruiting bodies in which a small sporophyte has developed by the division of the oospore into a number of cells. Note the advance of the sporophyte over that of *Oedogonium*. How many cells does it contain?

9. Each cell of the small, oval sporophyte develops a zoospore, which after a period of activity settles down and develops into a new *Coleochaete* thallus. It is evident from the above that the entire sporophyte of *Coleochaete* is sporogenous.

10. Make a diagram in the notes, showing the life cycle of *Coleochaete*. See Fig. 6.

11. If material is at hand study the flat, disc-like thallus of *Coleochaete scutata* Bréb. Draw under high power and describe.

12. Note. The *Coleochaete* are the algæ which come nearest to the next higher sub-kingdom, and, on account of the similarity of the body and the life cycle, the ancestors of the lowest liverworts of the present time are supposed to have been plants which in general characters were very much like them.

## Series II. ARCHEGONIATA. Sub-kingdom, BRYOPHYTA.

XLIV. (a) *Riccia fluitans* L. Class, Hepaticæ. Order, Marchantiales. Family, Ricciaceæ.

This liverwort has a small, linear, dichotomously branched thallus which grows floating in ponds and ditches. It also grows in wet places upon the ground, sometimes in cultivated fields. The plant keeps well along with other hydrophytes in a covered, glass jar of water.

1. Mount a small thallus or frond (gametophyte) in water and examine under dissecting microscope. Make a sketch of the plant and describe.

2. Draw a branch of the thallus under low power, showing the air cavities and cellular structure. Note that this thallus is not made of branching or interwoven filaments, but that it is a true solid aggregate. Most of the thallophytes are either simple or complex linear aggregates.

3. The aquatic form of this plant is usually sterile. In order to study the sexual organs and sporophyte to advantage, examine prepared slides of *Riccio-carpus*.

(b) *Riccio-carpus natans* (L.) Corda.

This plant forms a small, obcordate thallus which floats on the surface of ponds and swamps. The individuals are unisexual and develop the reproductive organs in late summer and autumn.

1. Sketch the living plant under dissecting microscope. Describe.

2. Under low power draw part of a section from a prepared slide, showing an antheridium. Draw part of a section, showing the archegonium containing the oosphere.

3. Draw an enlarged archegonium with the spherical sporophyte, containing a wall one layer of cells in thickness, with the spore-mother-cells (sporocytes) lying free in the interior.

4. From older stages draw spore tetrads and mature spores under high power.

5. If prepared slides are not at hand, cut freehand sections (with the aid of strips of fresh carrot roots) of male and female plants and draw an antheridium and an archegonium under low power. Also cut sections of a female plant containing a sporophyte and some free spores.

6. Compare the sporophyte with that of *Coleochæte* and note the beginning of sterilization of the tissue of the sporophyte.

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## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Davis, Bradley Moore. Oogenesis in *Saprolegnia*. Bot. Gazette, **35**: 233-249, 320-349, pls. 9-10, 1903. Published also in Decennial Publications, The University of Chicago. Ser. I, **10**: 225-257, 1903.

The original collection bore oogonia with few antheridia, but by cultivating on a rich substratum—raw beef or fresh insects—the antheridial filaments

become more numerous. On boiled eggs and dried beef oogonia are formed in great numbers, but there are no antheridia. After a time all cultures cease to bear oogonia and antheridia and produce only zoosporangia. Oogonia can always be produced by placing cultures with zoosporangia in such conditions that the hyphæ are not submerged. This can be done by placing material on cold agar-agar, where the filaments out of water soon develop oogonia.

In fixing, 1 per cent. chromo-acetic acid caused immediate contraction of the protoplasm. A solution of  $\frac{1}{4}$  per cent. chromic acid and  $\frac{1}{10}$  per cent. acetic gave the best results, having advantages over Flemming's, Merkel's, corrosive sublimate, sublimate acetic, iridium chlorid or picric acid. Safranin and gentian violet were used for staining.

The study of oogenesis was made upon apogamous material of *Saprolegnia mixta*. The resting nucleus has essentially the same structure as in the higher plants. In the cœnocyctic oogonium there is one simultaneous mitosis, the spindles being intranuclear. There are four chromosomes and no centrosomes. The differentiation of the egg origins (*Anlage*) takes place around cœnocentra which exert a chemotactic influence upon the nuclei in their vicinity. Generally, one nucleus lies close to the cœnocentrum and increases in size while all other nuclei in the egg origin degenerate, so that eggs are usually uninuclear. Trinucleate eggs are sometimes found and binucleate eggs are not uncommon, but the binucleate condition need have no relation to sexuality. In the formation of eggs, the protoplasm collects in denser masses about the cœnocentra with their accompanying nuclei. The protoplasm between the egg origins becomes less dense and a series of vacuoles appears which separate the origins from each other and allow them to round off as independent eggs.

The investigations upon sporogenesis resulted in a general confirmation of the accounts of Rothert, Hartog and Humphrey.

More than half of the paper is devoted to a theoretical discussion of homologies and relationships among the Phycomycetes and Ascomycetes. The oogonia and antheridia of Peronosporales, Saprolegniales and Pyronema are the homologues of gametangia and of the cœnogametes of Mucorales. In the evolution of the cœnogamete, eggs with a larger number of nuclei are regarded as more primitive, there being a tendency to reduce the number of nuclei, rather than the reverse. The Mucorales, Peronosporales and Saprolegniales can be

related to each other only through a common ancestry whose sexual organs were cœnogametes. The Ascomycetes may represent two evolutionary lines derived from a primitive cœnocyctic type of sexual organ (cœnogamete). C. J. C.

**Lawson, A. A.** On the relationship of the nuclear membrane to the protoplast. Botanical Gazette, **35**: 305-319, pl. 15, 1903.

The spore mother-cells of *Passiflora caerulea* and the archesporial cells of *Equisetum limosum* were especially studied, but observations were also made upon *Lilium*, *Cobæa*, *Gladiolus*, *Hedera*, *Pinus*, *Pteris* and others. For comparison, several members of the Cyanophyceæ and lower Chlorophyceæ were investigated. The principal conclusions are as follows: The typical nucleus of higher plants is a water cavity structurally similar to a cell vacuole. Chromatin is the only permanent constituent of the nucleus. The karyolymph, linin, nucleoli and membrane are renewed at each mitosis. The nuclear membrane is formed by the cytoplasm coming into contact with the karyolymph just as the tonoplast of an ordinary vacuole is formed by the cytoplasm coming into contact with the cell sap. The nuclear membrane is of cytoplasmic origin and should be regarded as the inner limiting membrane of the cytoplasm rather than as a constituent of the nucleus. In the Cyanophyceæ and Bacteria there are chromatin granules which represent the nucleus although they are not surrounded by any membrane or karyolymph. In the forms of chlorophyceæ which were examined, the karyolymph and nuclear membrane were uniformly present.

Flemming's stronger solution, diluted one-half with water, was used for fixing and the safranin, gentian-violet, orange combination for staining. Sections were cut 1-3.6 $\mu$  in thickness. C. J. C.

**Wisselingh, C. van.** Untersuchungen über *Spirogyra*, Vierter Beitrag zur Kenntniss der Karyokinese. Bot. Zeit. **60**: 115-138, pl. 5, 1902.

In the previous papers of this series the writer has devoted his attention to the nucleolus and the nuclear network.

The present contribution deals with the nuclear membrane, the spindle and the behavior of the walls of the vacuoles. *Spirogyra triformis*, a form with thin walls and loose, delicate chromatophores, was chosen for study. Material was fixed in Flemming's solution, after which it was treated with a strong chromic acid solution (40 per cent.) which dissolved successively the cytoplasm, karyoplasm and nucleolus. The spindle, however, was not dissolved. Sections do not seem to have been used.

During the earlier stages of karyokinesis the nuclear membrane is entirely resorbed. The spindle is derived from the granular cytoplasm about the nucleus and consists of but one kind of fibers, the two different lengths of fibers and the two opposite groups described by Strasburger for *S. polytenuata* not appearing in *S. triformis*. The spindle fibers do not grow through the nuclear membrane as described by Strasburger. The spindle is at first multipolar, but becomes bipolar. There is no diminution in the number of spindle fibers during karyokinesis, but after karyokinesis the spindle becomes resolved into cytoplasm. The spindle fibers resist the action of chloral hydrate and so are easily distinguished from cytoplasmic strands. The walls of the vacuoles are also made visible by chloral hydrate. During karyokinesis the walls of the vacuoles with



some cell sap press between the spindle fibers and appear within the spindle. Between the two halves of the nuclear plate a number of plasma strands are formed enclosing the spindle fibers, but there is no persistent, closed connecting tube as described by Strasburger for *S. polyteniata*.

C. J. C.

**Allen, Charles E.** The early stages of spindle-formation in the pollen mother-cells of *Larix*. *Annals of Botany*, **17**: 281-312, pls. 14-15, 1903.

Material of *Larix europæa*, DC was fixed in various solutions, of which Flemming's proved most satisfactory. Collections were made in autumn, winter and spring.

Late in October the pollen mother-cells are easily distinguishable, but division does not occur until the following spring. The earliest stages in the development of the spindle are described in great detail. The writer describes five stages in the formation of the spindle, viz., the pre-radial stages, the radial stages, the formation of the felt, the multipolar spindle, and the completion of the spindle. The conclusion is drawn that, from the very early prophases, there is present in the cytoplasm a distinct fibrous system which, in conjunction with another set of fibers of nuclear origin, forms the spindle. The fibers of an early reticulum become arranged into a radial system, and this in large part passes into an extra-nuclear felt, and the fibers of the felt form the extra-nuclear part of the spindle. The fibers are something more than lines of force or expressions of strains or stresses. They are organs with distinctive chemical and physical properties which determine their power to do particular kinds of work.

No centrosomes were observed at any stage in mitosis.

C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

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Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole, 55 S. Marengo Avenue, Pasadena, Cal.

**Wolff, E.** Beobachtungen bei der Färbungen der elastischen Fasern mit Orcein. *Centralbl. f. allgem. Pathol. u. pathol. Anat.* **13**: 513-518, 1902.

The author found that orcein solutions originally actively staining became inactive, experiment showing that it

is essential to have the solution exposed to air and light. Also the orcein must acquire a certain ripeness, and with these two conditions fulfilled the stain keeps good indefinitely. Different kinds of orcein were used, giving various results. Of Gruebler's orceins, orcein d ripened the most rapidly, 8 days; orcein a required 4 weeks, orceins b, c, d were intermediate. The solution is used as follows: a stock solution is made in 90 per cent. alcohol; when ripened, sufficient of this is put into 70 per cent. hydrochloric acid alcohol to make a solution of a wine red color. The tissue to be stained is put into a closed vessel with the stain for 24 hours, longer if the tissue stains with difficulty. With a weak solu-

tion it is almost impossible to overstain; if this occurs, acid alcohol readily removes the superfluous amount. Then wash in distilled water, counterstain, etc., if desired. The stain is the same, whether the material is old chromic, or acid, or formalin hardened. At the most a longer time, 48 hours or so, is required in these cases. Any method of embedding can be used. Celloidin is good, but especially fine results come from frozen sections, stained in orcein. Through evaporation of the alcohol of the stain on the section a rapid coloring of the connective tissue fibers results. The author counterstained with hematoxylin, alum hematoxylin. Twenty-four hours in a weak or a shorter time in a strong solution stains sufficiently. With celloidin after the latter stain, treat with acid alcohol, wash in tap water till again blue. If thionin is used for contrast stain, a hot saturated aqueous solution is best. Filter as much of the latter into distilled water as will make a blue mixture, too dark to distinguish the sections through, wash the alcohol off and stain from a few minutes to an hour. This stain is only permanent if the sections are mounted in resin. A small piece of the latter is melted on a slide, the section laid on a cover-slip in xylol, dried with tissue paper and laid on the resin, which meanwhile has cooled sufficiently to avoid injury to the section and yet remained fluid enough to use. Air bubbles may be troublesome at first, but a little practice does away with them. The counterstain with carbol toluidin is admirable. A concentrated alcoholic solution is best. Enough of this is dropped into a 2 per cent. carbolic acid solution (aqueous) to make a dark or light blue color, for rapid or slow (24 hours) stain. Wash with distilled water and alcohol, differentiate in beechwood creosote if necessary, clear in xylol and mount in balsam; a permanent stain results. This is especially good for mast and plasma cells; after 24 hours staining and final warming, the different micro-organisms, the pus microbes, pneumo-, staphylo-, strepto-, gono-, and meningococci, as well as coli, typhoid and influenza, are stained. Owing to their dark blue color the bacteria stand out clearly from the rest of the tissue. In addition to these results, Weigert's fibrin and tubercle stain can be got. The tissue is stained in hematoxyn after being well washed, sections are treated with carbol fuchsin and then with Weigert's fibrin stain. A. M. C.

**Mac Cullum, W. G.** On the relations of the Lymphatics to the Peritoneal Cavity in the Diaphragm and the mechanism of absorption of granular material from the Peritoneum. *Anat. Anzeig.* 157-159, 1903.

From the study of the diaphragm of dogs the author concludes that the peritoneal epithelium is a complete and unbroken layer of cells without pre-formed stomata in the sense of von Recklinhausen. The cells forming this layer have the power of retracting slightly from one another, the lymphatics of the part form a dense layer beneath the epithelium, the radial trunks embedded in the musculature and abundantly connected with the pleural network are connected by arching transverse anastomose, which lie in the superficial connective tissue and then come close to the peritoneal epithelium. These "lacunæ" have only a thin roof of peritoneal epithelium, the lymphatic endothelium and a lattice work of fibrils separating them from the cavity. The endothelium, as demonstrated by recognized methods, forms an unbroken membrane. Injection of the lacunæ with colored masses tends to prove the completeness of their walls; they may

be widely distended without leakage. Granules injected into the peritoneal cavity enter the lymphatics of the diaphragm in great quantities and appear in the mediastinal lymph glands. In the living animal after a short time the peritoneal fluids are found swarming with phagocytes laden with granules, they are found to make their way between the cells into the lymphatics from whence they are swept away to the gland sinuses. Movements of respiration but slightly affect the process. Granules can enter the lymphatics without the aid of leucocytes, as shown by using the dead animal, but their number is far less; yet the mechanical factor concerned in the process cannot be entirely eliminated. In such a diaphragm the granules lie about the cell borders, outlining them much as does silver nitrate. They may be seen along the retracted margins of the cells, filling the intercellular spaces; finally they reach the lumen of the lymphatics. In embryonic life there is a time when the pleural and peritoneal spaces are completely separated by the diaphragm, later the lymphatics enter this part and intimate relations arise. This shows that the peritoneum is not a mere dilatation of the lymphatic system. The fact is especially brought out by these results that the lymphatics are here, as in almost all other places, a closed system of canals distinct from the connective tissue and from the peritoneal epithelium.

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

Lendenfeldt, R. von. Eine biologische Notiz über *Spongilla fragilis* Leidy. Arch. f. Naturgesch. 69: I, 181-182, Taf. X, 1903.

Living sponges with gemmulæ collected October 9th were placed in an aquarium with running water, but within three days they all died. The gemmulæ were then found upon the glass sides of the aquarium, where the growth of the young sponges had already begun. By the 24th they had formed lobed patches 3 cm. in diameter, remaining without change in the arrangement of the canal systems until the 26th, when gemmulæ began to form. On the 27th the canal systems began to diminish and the soft tissues to fall away, the process continuing rapidly until the 30th, when gemmulæ formation was completed. Bent spicules were found to be more abundant in sponges grown in aquaria than in natural conditions in the stream.

C. A. K.

Isert, A. Untersuchung über den Bau der Drüsenanhänge des Darms bei den Monaciden. Arch. f. Naturgesch. 69: I, 237-296, Taf. 12-15, 1903.

Material from the Adriatic and Mediterranean seas was fixed and preserved in 10 per cent. formalin. Alcoholic material was also used. Diluted alum carmine was employed for *in toto* staining, and Delafield's hæmatoxylin and orange G on sections.

C. A. K.

Schaffer, J. Versuche mit Entkalkungsflüssigkeiten. Zeitsch. f. wiss. Mik. 19: 308-328, 441-463, 1903.

The author has made an exhaustive series of experiments to determine the most satisfactory reagent for decalcifying vertebrate tissues and the most successful method of applying it. He has tested practically all of the acids recommended for decalcifying in various strengths, and in some cases in combination with alcohol or phloroglucin. He has also determined the best method of removing the acid from the preparations. The most complete decalcification with a minimum of deformation due to shrinkage, swelling, or dissolution of the tissue, was obtained by carefully embedding well-fixed tissues in celloidin, hardening the celloidin block in 85 per cent. alcohol, grading down through lower per cents. of alcohol to water, exposure for 12 to 24 hours (longer for large masses of tissue) to 3 to 5 per cent. (by weight) aqueous solution of nitric acid in a Thoma's water wheel. From the acid the block is passed to a 5 per cent. solution of lithium or sodium sulphate for 12 to 24 hours. This solution should be changed at least once. The sulphate is removed by washing for 48 hours in running water, after which the object is again graded up to 85 per cent. alcohol.

Phosphoric, lactic, formic, and acetic acids, even in concentrated solutions, were found to produce swelling in tissues containing collagen, and their dissolving power is also so slight as to preclude their use in decalcification. Formic or acetic acid may, however, be used in connection with reagents which prevent swelling, or on tissues fixed in osmic acid or formalin, but never on fresh tissues or on those fixed by ordinary methods.

The author finds that the following acids are available for use on heavily calcified tissues, such as bones and teeth; viz., hydrochloric, nitric, trichloroacetic, and sulphuric, named in order of their decalcifying capacity. Swelling is caused by the trichloroacetic and still more by the sulphuric acid, increasing after washing out the acid with the first-named, but diminishing after the last. Both produce precipitates in the tissues, which are, however, easily soluble in water and therefore necessitate careful washing out after decalcification. This second group of acids produce no swelling in tissues fixed in formalin when they are used in solutions of 3 to 10 per cent. Long action of the hydrochloric acid affects the chromatin and reduces its stainability. For this reason nitric acid is to be preferred to the hydrochloric. Its dissolving power is but a little less than that of the hydrochloric acid, and it leaves the tissues, cells and nuclei in much finer condition, and retains their elective coloration.

The nitric acid may be used without damage to tissues in strengths of 2 to 10 per cent., but since 10 per cent. or even 20 per cent. decalcifies no more rapidly than 5 per cent. it is unnecessary to exceed this strength. This 5 per cent. aqueous solution (by weight) will decalcify without trace of swelling 42 grams of solid bone in 10 hours. Additions to the nitric acid tend to prolong the decalcification, phloroglucin but slightly, formalin still more, and alcohol most of all. Low grades of alcohol not only tend to prolong the decalcification but also swell and shorten the fibrillæ. Five per cent. alum solution does not affect the rapidity of action of the acid, but otherwise furthers the decalcification. All these additions to the acid have been employed to prevent swelling, but the 2 to



5 per cent. nitric acid causes no swelling. This appears only as the acid is diluted in washing out and may be prevented by using formalin or alum as a medium for the acid, but not by phloroglucin or alcohol. If aqueous solutions of the acid are used the neutralizing agent should not be alcohol, formalin, salt solution, or phloroglucin. Five per cent. solution of potash alum may be used since it causes only a temporary swelling, but the best agent is 5 per cent. lithium or sodium sulphate, renewed frequently with large objects and thoroughly washed out afterwards in running water.

C. A. K.

**Wacke, R.** Beiträge zur Kenntniss der Trematoden. Zool. Jahrb. Suppl. vi. Fauna Chilensis 3: 1-117, Taf. 1-9, 1903.

These parasites were secured from fresh water or land Crustacea in Chili and New Zealand. After treatment in cocaine they were fixed in alcohol or in chrom-osmic-acetic. Histological details were determined on individuals stained *in toto* in Delafield's hæmatoxylin, alum-carmin, borax-carmin, picro-carmin or picric acid. Sections 5 or 10 microns in thickness were cut, and teasing by the microtome knife was prevented by coating the sections with mastix-collodion. A triple stain of hæmatoxylin-eosin-orange G, in sequence of naming, was used. Sections were mounted in balsam or carbol-glycerine and histological details were found to be sharply differentiated. Total preparations were mounted in clove-oil or carbol-glycerine. The author concludes that this puzzling group of ectoparasites forms a connecting link between the rhabdocœl turbellarians and the trematodes.

C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory, University of Michigan, Ann Arbor, Mich.

**Sajous, C. E. deM.** The Internal Secretions and the Principles of Medicine. Vol. 1. With forty-two illustrations. Philadelphia, F. A. Davis & Co. 1903. Pp. xxvi and 800.

This great work of Sajous', of which only the first volume has so far appeared, has for its purpose the presentation, elaboration, defense, and application to medicine of a theory of the physiology of the animal body. That the theory is original, even startlingly so, may be seen from the following very brief summary quoted from the preface: "Briefly, our inquiry seems to us to have shown that the adrenal system" ("Adrenal system" is the term used by the author to include the thyroid gland, the anterior pituitary body and the adrenals) "is the source of the secretion which, with the oxygen of the air, forms the oxidizing substance of the blood-plasma. It has also revealed, we believe, the origin and mode of distribution of the bodies with which this oxygen directly or indirectly combines: i. e., peptones, myosinogen, fibrinogen, hæmoglobin, and myelin" (peptones, myosinogen, and fibrinogen formed by neutrophiles, hæmoglobin by eosinophiles, and myelin by basophiles) "to insure the continuation of life and the efficiency of all organic functions. Finally, it has suggested that in addition to these agencies, all leucocytes and, under certain circumstances, the plasma, contain a protective agency, trypsin, which, with

Metchnikoff's phagocytic cells, serves to destroy micro-organisms and convert their toxins and other albuminoid poisons into harmless products. Considered jointly, these various factors seem to us to represent the aggregate of vital phenomena." Such, in barest outline, is the standpoint of the work. Some idea of its scope may be gained from the chapter headings. These are: I. The Physiology of the Adrenals as Viewed from the Standpoint of Clinical Pathology. II. The Internal Secretion of the Adrenals in its Relations to the Respiratory Processes and the Composition of the Blood. III. The Internal Secretion of the Adrenals in its Relations to the General Oxidation Processes. IV. The Internal Secretions of the Thyroid and Thymus Glands in their Relations to the Adrenals. V. The Anterior Pituitary Body, the Thyroid Gland, and the Adrenals as Parts of an Autonomous System. VI. The Adrenal System and Vasomotor Functions. VII. The Adrenal System, the General Motor System, and the Pneumogastric Nerve. VIII. The Internal Secretions of the Pancreas and Spleen. IX. The Adrenal and Vagal Systems in their Relations to Cardiac and Pulmonary Functions. X. The Posterior Pituitary as the Functional Center of the Nervous System, and as the Anterior Pituitary's Co-center in Sustaining the Vital Processes. XI. The Internal Secretions in their Relation to Immunity. XII. The Internal Secretions and the Preservation of Life.

The work as a whole may be characterized as an analysis and subsequent synthesis, from the above quoted point of view, of a really enormous mass of the literature of physiology and pathology. In fact, practically the only evidence brought forward by Sajous in support of his theory is evidence gleaned from the literature, which, as every one knows, is not the best of material for constructive purposes, when one wishes to build up a sound hypothesis.

Leaving aside the question of opinion as to the value of the conclusions of this book, the reviewer has found it very interesting and suggestive reading. It will undoubtedly prove useful to all biologists, using the term in its widest sense, as a careful digest of a great mass of literature dealing directly and indirectly with the subjects under discussion. "Special pharmacodynamics and physiological pathology" will form the subject matter of the second volume of the work.

R. P.

**Knight, A. P.** Sawdust and Fish Life. Trans. The author has performed a number of experiments to settle the question of the effect on fish of the mill waste which is discharged into many freshwater ponds and streams. He found that concentrated extracts (aqueous) of white pine sawdust and of cedar sawdust were highly poisonous to various forms of aquatic life, including fish and a number of invertebrates. If the water lying above such a concentrated solution of sawdust and into which the solution has not diffused, is well aerated fish will live in it as well as in any water. The extracts from sawdust of other woods than pine and cedar, including maple, oak, ash, elm, hemlock, spruce, and others are much less poisonous. The author shows that a number of conditions must be taken into account and thoroughly understood before any given stream can be said to be poisoned with sawdust.

R. P.

Kahn, R. H. Die Bürstenwippe. Centralbl. f. Physiol, 17: 34-38, 1903.

Kahn has devised a new electric key and current reverser for use in physiological experimentation, which has certain advantages over forms now in use. The instrument is shown in plan in Fig. 1, and in perspective in Fig. 2. It is

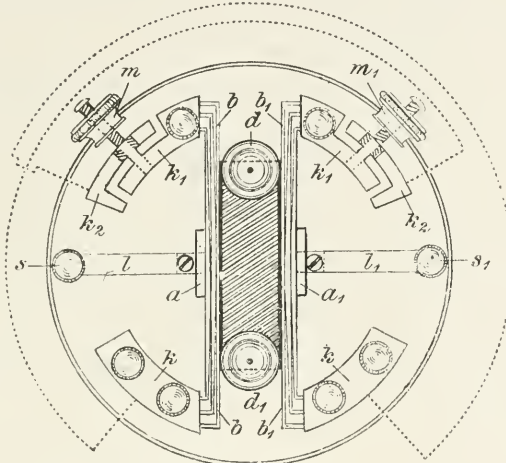


Fig. 1.

made by J. Krusich, Universitätsmechaniker, in Prag. In the center of a hard rubber plate about 80 mm. in diameter are two metal brushes  $b\ b$  and  $b_1\ b_1$ , isolated from one another by a block of hard rubber. The ends of the brushes are raised from the horizontal, so that when one end makes contact the contact

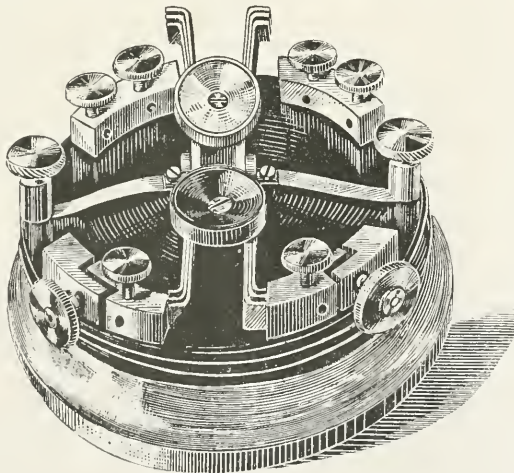


Fig. 2.

at the opposite end is broken. The ends thus have a range of movement of  $25^{\circ}$ – $30^{\circ}$ . When either end is depressed it makes, on account of the spring in the brushes, a firm contact with one set of metal blocks, either  $k-k$  or  $k_1\ k_2$ – $k_1-k_2$  as the case may be. Movement of the brushes is brought about by

pressure on the buttons  $d$   $d_1$ . Connection is made from the brushes, through the axis plates  $a$   $a_1$  and the metal strips  $t$   $t_1$ , to bonding posts  $s$   $s_1$ . The contact blocks  $k$   $k$  are solid, while  $k_2$   $k_2$  are loose metal blocks which can be brought into contact with  $k_1$   $k_1$  by means of the milled heads  $m$   $m_1$ . Each  $k$  block is cross connected by a metal strip (represented in Fig. 1 by the dotted lines) with the  $k_2$  block on the opposite side of the instrument.

As will be seen from the diagram, the instrument may be used either as a simple key or as a current reverser, and in fact for all the purposes to which a Pohl commutator is adapted.

R. P.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn, Wesleyan University, Middletown, Conn.

**Nebel.** Ueber den Nachweis der Tuberkelbacillen im Sputum. Arch. f. Hyg. xlvii, p. 57, 1903.

improvement in methods of great value. Nebel has devised a means of more accurate and simple examination of sputum which contains only a small quantity of tubercle bacilli. Sputum is placed in a wide mouthed bottle, closed with a rubber stopper and thoroughly mixed by shaking with ten times its bulk of lime water. It is then centrifugalized for two minutes. The effect of the centrifugalization is not to separate the bacilli from the liquid, but makes their subsequent detection easier. The liquid is separated from the sediment by a pipette and placed in a Berkefeld-filter beaker, holding about 15 c. c. This in turn is placed in a large beaker filled with loose dry gypsum. The liquid filters through the beaker in two to three hours and is absorbed by the gypsum. The filtrate may then be removed with a platinum needle, placed in a drop of water and stained on a cover-glass in the usual way. The advantage of the method is that the bacilli are concentrated in the material that fails to pass through the filter. The method of testing is said to be rapid, cheap and efficient.

H. W. C.

**Savrases.** Colorabilité des bacilles de Koch dans les crachats incorporés à diverses substances. Ann. d. l'Inst. Past. xvii, p. 301, 1903.

The question as to whether tuberculous sputum after disinfection by various means is still capable of study for the detection of the bacilli has been investigated by the author. Recognizing the occasional need for such a test he has attempted to determine whether the materials commonly used for disinfection of sputum so destroy the bacilli that they cannot be subsequently detected. His method is to mix the saliva with a certain quantity of the disinfecting material and leave it for about forty-eight hours, and then to test in the ordinary manner. He tests the action of distilled water, boiling water, glycerine, alcohol, chloroform, benzine, carbolic acid, sulphate of soda, bichloride of mercury and a long series of other agents. His conclusion is that the action of most of these agents is inefficient to prevent the typical

The great practical value of the microscopic examination of sputum for the detection of tubercle bacilli makes any

The question as to whether tuberculous sputum after disinfection by various means is still capable of study for the



staining of tubercle bacilli by the Ziel-Neilsen method and that sputum after disinfection may be thus microscopically tested.

H. W. C.

**Rosenau.** An investigation of a Pathogenic Microbe applied to the destruction of Rats. Bul. V. U. S. Marine Hospital Service, 1901.

The use of of a pathogenic bacterium, *B. Typhi murium*, for the destruction of rats was advanced by Loeffler some

years ago and has been used with more or less success. Rosenau has experimented as to methods of culture and use and has determined the interesting point that if this bacterium is cultivated in contact with air it gradually loses its pathogenic properties. If, after such cultivation, it is distributed so that rats feed upon it, the rats are not injured by it but are actually rendered immune, thus becoming protected from subsequent virulent cultures. Rosenau insists, therefore, that the cultures used must be of the highest virulence or their distribution will not only be useless but will actually render the animals more resistant against any further attempts at their destruction. He regards the use of the bacillus as preferable to the use of poisons for destroying rats inasmuch as this organism is harmless to man.

H. W. C.

**Lehmann.** Beobachtungen über die Eigenbewegung der Bakterien. Arch. f. Hyg xlv, p. 311, 1903.

No efforts have hitherto been made to determine the actual rapidity with which motile bacteria can move,

although the difference in velocity of different species is a matter of common observation. Lehmann measures the velocity of movement by the simple means of direct observation. The vigorous culture of the organism is placed in a hanging drop and brought under the microscope. In the eyepiece is placed a micrometer eyepiece, so adjusted that each division of the scale corresponds to a known distance upon the slide. He then selects single bacteria which is in rapid motion and determines the number of seconds required for it to pass the distance from one division in the micrometer to the next. A large number of such observations were made upon different individuals of the same culture and then an average of the whole taken as the mean rapidity of motion. He found that the cholera bacillus moves most rapidly of the species studied, moving at the average rate of about one mm. in  $34\frac{1}{2}$  seconds. *B. megatherium* moved the slowest, moving about one mm. in 2 minutes and 11 seconds.

H. W. C.

**Lindner.** Der Tuschpinsel und seine Verwendung bei Anlage von platten kulturen, zur, "Pinselstrichkultur." Woch. f. Brauerei, xx, p. 57

Lindner believes that the use of a brush is a great convenience in studying the relative number of bacteria in contaminated water and similar fluids

and especially in determining whether they are increasing or decreasing. He sterilizes the brush, either in heat or by soaking in 94 per cent. alcohol, and dips it in the liquid to be tested. Then he brings it into a tube of sterile water and, after thorough washing and agitation, he draws the brush, taken from the diluted mixture, over the surface of the Petri dish containing hardened culture medium. The brush is then brought into a second tube of sterilized water and the bacteria remaining on the brush thus diluted a second time in water. In the same way a second streak of inoculation is made, and a third dilution in the same

way. The relative number of bacteria may be easily determined in this way, but evidently no actual quantitative determinations are possible. H. W. C.

**Baermann.** Ueber die Zuchtung von Gonokokken auf Thalmanschen zw. gewöhnlichen Fleischwasseragar und Glycerine agar-Nährboden. Zeit. f. Hyg. XLIII, p. 529, 1903.

The culture of *Gonococcus* has presented great difficulties and a successful method of growing it is a great desideratum for pathologists. Thalmann has prepared a culture medium which he regards as especially useful. It consists of the common bouillon agar with slight modifications. The ground beef is dissolved for fifteen minutes in boiling water and a small sample is neutralized, with phenolphthalein as an indicator, until there is a permanent red color produced. From the amount of NaOH necessary for this neutralization is calculated the amount needed to similarly neutralize the whole amount, and then 70 per cent. of this amount is added to the whole medium. Baermann tests this medium and compares it with ordinary media with the following results: He finds that no cultures can be obtained with any media unless there is a rich sowing of pus, but with such sowing cultures can be obtained upon common agar as well as glycerine agar. The Thalmann's medium is somewhat inferior to common flesh agar. There are many unaccountable differences in the results obtained with the same medium in his different tests. H. W. C.

**Besancon, F., Griffon, V. and Philibert.** Comptes Rendus de la Société de Biologie. Number 1, January, 1903.

The writers of this article advance the following method of determining the presence of the bacillus of Koch in the blood:

"Place five cubic centimeters of blood in a mortar (both clot and serum). Add five cubic centimeters of distilled water, and five drops of sodium carbonate solution. Triturate the clot until it dissolves in the liquid. To this mass add twenty cubic centimeters of water and boil in an evaporating dish for ten minutes.

"Pour the liquid into two tubes of a centrifuge of rapid rotation, and turn rapidly for about ten minutes. The deposit obtained should then be placed upon foil and treated according to Ziehl's method."

In this way, these writers have been able to identify the bacillus of Koch by a microscopical examination of the blood of animals inoculated with tuberculosis for experimental purposes (two rabbits and one guinea pig having been inoculated hypodermically the day before), and in human blood infected with tuberculosis (one case of pulmonary tuberculosis and one of plasmic pleurisy).

In a second communication made to the same society (Number 5, February 1903, pages 203 to 204) the same investigators admonish against a "possibility of error in the diagnosis of the tubercle bacillus based upon the microscopical examination of blood clots." They state that in case the blood or the suspected serum has not been taken with the greatest aseptic precautions, no one has a right to consider as a bacillus of Koch every bacillus retaining the stain after the action of Ziehl's solution and the decoloration by a twenty-five per cent. solution of sulphuric acid.

A. GIRAULD.

## GENERAL LABORATORY TECHNIQUE.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory,  
University of Michigan, Ann Arbor, Mich.

### The Use of Clay Modeling in the Study of Osteology.

Dr. R. O. Moody describes in the Bulletin of the Johns Hopkins Hospital (Vol. xiv, pp. 78-81) the results of his

experience in having students of human osteology make clay models of bones as a part of their laboratory work in the subject. He commends the plan highly on pedagogical grounds, and gives a very interesting and suggestive account of how the work is carried on in the Medical Department of the University of California. In this work the "outfit of the student consists of a paraffined board and two modeling tools, Boxwood 64 and E. & B. No. 3. The first of the instruments may be easily made by any one who can use a jack-knife, and the second by any one who has in addition a little manual dexterity. The board should be a piece of kiln-dried finished wood about 60 centimeters long, 50 centimeters wide, and 2 centimeters thick. It is well to have it coated with boiling paraffin to prevent the clay from sticking or the board from warping. We have found it desirable to use the best potter's clay that can be obtained. This should be free from stains and foreign bodies. It is prepared by grinding it into a fine powder and, after passing it through a sieve, adding a sufficient quantity of water. This mode of preparation not only removes foreign bodies but permits the water to act evenly on the clay. It is important to have the two homogeneously mixed. Clay that is more moist in some parts than others is difficult to mold and liable to crack when dry, thus destroying the model. It is ready for use when it contains enough water to mold easily but not sufficient to make it sticky. When there is a little too much moisture, kneading will often bring it to a proper consistency, but if it is very wet and sticky some powdered clay may be added to take up the excess. In this case, however, the kneading should be thorough in order that the clay shall have throughout a uniform moisture. When given to the students, the clay should be in the proper state of plasticity for immediate use, but it is well, however, to have the student thoroughly work it over with his hands, for the better the clay is worked, the more readily it can be molded. The bones should be completed while the clay is still in a plastic condition. This state can be maintained indefinitely by covering the model with wet cloths during the various stages of its preparation. There is a notable tendency among the students, especially before they have learned how to handle soft clay, to outline the bone roughly, let the clay harden, and then carve and finish the model. This, however, is unsatisfactory, as the student who carves loses the training of the tactile sense and often cannot make the necessary changes which the instructor suggests to improve the model or make it conform more closely to the original specimen. In fact, the less modeling the student does with his tools and the more he does with his fingers, the greater

will be the benefit of the work to him. Most of the bones are modeled as isolated objects, but some of them, preferably the more fragile ones, like the fibula, may be modeled on a base. The skull, the wrist and hand, and the ankle and foot are usually modeled on a base. To model some of the long bones which have naturally only one or two points of contact with the underlying board it is often necessary to support the shaft at short intervals by clay pillars, which remain under it until the model is dry. When the student has completed a specimen the original bone as well as the model is submitted to an instructor. This, too, should be done while the model is still in a plastic state, for a careful comparison of the model with the bone usually reveals the necessity of changes, sometimes quite fundamental. Not infrequently the student carries the modeling of one or two dimensions nearly to completion without due regard to the others. In order to produce good results it is naturally necessary to bear in mind constantly the relations of one to the other two dimensions. When the bone is finished and accepted by an instructor it is marked with the name or initials of the maker and placed with the remainder of his specimens. The general form and appearance of a model made on a base may often be brought into sharp relief by coating the base with shellac and then with enamel paint or by coating both base and model with shellac and paint of different colors for a contrast. If it is desired to make the models permanent they may be burned like pottery. At the end of the time scheduled for osteology a practical examination is given in which each student is required to model one bone and draw several others entirely from memory. The author exhibits photographs of samples of models made by his students, and so far as can be judged from these pictures, the work is very creditable, both to the students and to the method as a pedagogical discipline.

R. P.

#### The Metric Equivalents of Apothecary's Weights and Measures.

The following tables (from the Jour. Amer. Med. Assoc., March 1, 1902) for the conversion of apothecary's

weights and measures to their metric equivalents, may be found useful by laboratory workers in general.

TABLE OF WEIGHTS.

1 grain	-	-	-	-	-	.065 gram
15.43 grains	-	-	-	-	-	1 gram
1 dram (troy)	-	-	-	-	-	3.90 grams
1 ounce (troy)	-	-	-	-	-	31 grams

TABLE OF CAPACITY.

1 minim	-	-	-	-	-	.061 c. c.
16 minims	-	-	-	-	-	1 c. c.
1 fluid dram	-	-	-	-	-	3.75 c. c.
1 fluid ounce	-	-	-	-	-	30 c. c.

Thus, to convert grains or a fraction of a grain into the corresponding quantity in the metric system, multiply the number of grains by .065, and to convert the quantities written in the metric system into their equivalents in grains, divide by .065.



To convert drams into grams, multiply the number of drams by 3.75, and to convert grams into drams divide the number by 3.75, or less approximately and more conveniently by 4. The same rule is to be observed in dealing with ounces. But in converting minims, as observed in the second table, into the metric system the number of minims must be multiplied by .061 c. c., the number of fluid drams must be multiplied by 3.75 c. c., and the number of ounces by 30 c. c.

R. P.

### Waterproof Cement for Glass.

In making pieces of apparatus for experimental work, in repairing or remodeling aquaria and for various laboratory practices it is frequently desirable to have a cement which will hold pieces of glass together firmly, and at the same time will not be affected by water. The Scientific American (February 28, 1903) gives some useful formulæ for such cements. These are:

1. Dissolve 5 to 10 parts gelatine in 100 parts water; add 10 per cent. of a concentrated solution of bichromate of potash, mix thoroughly and keep in a dark place. When the articles joined by this cement are exposed to sunlight for a short time, the cement becomes tough and insoluble in water.

2.	Quicklime	-	-	-	-	-	4 parts
	Litharge	-	-	-	-	-	6 "
	Linseed oil varnish	-	-	-	-	-	1 part

R. P.

### Staining and Preservation of Urine Sediments.

Kozlowski (Das Konservieren und Färbn von mikroskopischen Präparaten der Harnsedimente. Virchow's Arch.

169: 161) centrifugates the urine with about one-tenth part (volume) of a 1 per cent. eosin solution. After separation of the fluid a drop of the sediment is mixed on a slide with a drop of Farrant's medium (water, glycerine and a cold saturated solution of arsenious acid in equal parts, to which is added as much gum arabic as will dissolve) and covered with a cover-glass. The mounts should be sealed with some good cement. Preparations so made will hold the stain and not undergo any essential change for at least five years.

R. P.

## QUESTION BOX.

Inquiries will be printed in this department from any inquirer.  
The replies will appear as received.

No. 31.—Will some one give a description of a small aquarium for a private laboratory to secure specimens for demonstration in high schools, etc., and the best method to sustain life by isolation of species, gravel or mould aeration by water, etc., etc.?

B. E. B.

No. 32.—What is the best food or manner of treating biology specimens to keep them alive, as used in Huxley & Martin's Biology?

B. E. B.

No. 33.—Please describe how in a room for developing—with window covered with an extra sash of red and yellow glass in upper and lower sash—ventilation can be secured in summer and not admit the light.

V. A. L.

No. 34.—Will some resident in Chicago kindly give some directions as to *where* to seek biologic material—preferably on the north side district? I understood the park tanks were very prolific in micro forms, etc., but from whom should permission be obtained to study the infusoria, diatoms, etc.? The local society not being in existence any more, new residents are not up to the fauna localities.

B. K. T.

#### ANSWER TO QUERY 25, JUNE, 1902.

Ferns show a great variety of scales, sufficient to give the student occupation for a long time. On the underside of the leaves are the reservoirs for the spores, resembling green velvet, arranged in stripes, masses, and various other forms. The spores are usually covered by a thin skin and their manner of arrangement, changes and developments are an endless study, different ferns giving us many variations in this respect totally invisible without the microscope. The hymenophyllums (of which two only, I believe, belong to England) are particularly interesting. The leaf structure when dried makes them beautiful objects, often requiring no balsam to aid in translucency. Portions of the *fronds* of ferns should be mounted as opaque objects, after having been dried between blotting paper—avoid pressure. The time of gathering is an important factor, as they do not show their beauty before they are ripe, and if over-ripe the spore arrangement, etc., is altered. I find a preferable way to mount objects is one in which they can be studied as opaque or transparent when so desired. The spores can be mounted separately like pollen. Carefully pick and let dry in a moderately warm place in a pill box to keep free from dust; mount dry in a cell with a ring to hold the cover-glass up. Then coat over both ring and cover with gold size or brown varnish.

The under side of many ferns is covered with scales of very beautiful forms.

Fronds are mounted in rings as above described, and it is best to mount in various ways. The transparency from balsam interferes with one property of the object, and yet develops another which would have remained invisible if preserved dry. Many prefer the lieberkuhn for illuminating opaque objects; others place the slide on a wooden slip to make it opaque or transparent as desired; others again, by putting upon the under side of the slip, directly under the object, a spot of black varnish, which does not interfere materially with the light; by using some such method, space is reserved in one's cabinet. The lighter specimens can be mounted dry, the darker kinds in balsam. Staining can also be done if desired, magenta being one of the best vegetable stains.

The polariscope also aids in studying the forms of sori. To watch the development of the spores of ferns and the fertilization and products, the late Dr. Carpenter gave the following: Let the frond of a fern whose fructification is mature be laid upon fine paper with its spore bearing surface downwards; in a day or so the paper will be found covered with a very fine brownish dust, which consists of the discharged spores. Collect carefully; spread upon the surface of

a smooth fragment of porous soapstone; place the stone in a saucer, the bottom being covered with water; invert a tumbler over it, and the spores will grow well. Or place in the moist chamber given in the JOURNAL.

Some prothallia advance beyond the rest, produce antheridia and bear many archegonia. Now keep the crop with little moisture for several weeks, then suddenly water it and a great number of antheridia and archegonia simultaneously open, and in a few hours afterwards the surface of the larger prothallia will be found almost covered with moving antherozoids.

Such prothallia as exhibit freshly opened archegonia now hold by one lobe between the forefinger and thumb of the left hand, so the upper surface of the prothallium lies upon the thumb; take the thinnest possible sections perpendicularly to the surface of the prothallium. Some of these may lay open the canals of the archegonia and within these, when a power of 200 to 500 diameters is used, antherozoids may be distinguished.

The Prothallium of *Osmunda regalis* offers peculiar facilities for study. Mount in any of the following:

1. Carbolic acid, 1 drachm; alcohol, 2 drachms; dist. water, 12 ozs. Dissolve the acid with alcohol, add water, boil 10 minutes and bottle.
2. Acetate of aluminium, 1 part; dist. water, 4 parts. Is also used for algæ, etc.
3. Ralf's liquid, or Deane's compound.

For further notes, see Chamberlain's Notes in Botanical Methods, JOURNAL OF APPLIED MICROSCOPY; Strasburger's Practical Botany; Zimmerman's Practical Botany Technique; Huxley and Martin's Biology.

It is a good plan to secure a first-class mount from such workers as Miss M. A. Booth, Rev. J. W. King, and Miss Dewey to use as a sample of what to aim for.

V. A. LATHAM.

Rogers Park, Chicago, Ill.

---

The following is one of the best methods of staining the tubercle bacillus: The small, yellow, caseous-looking points from a sputum rich in the bacilli are spread out by pressure between two cover-glasses, so that a fairly thin film remains on each, when they are carefully slipped one over the other until they come apart. Thoroughly dry the covers, protecting them carefully from dust, pass rapidly three times through the flame of a spirit lamp, care being taken not to scorch the film, then float film-face downwards, on the staining solution, which has previously been filtered into a watch-glass. The stain should consist of saturated alcoholic solution of basic fuchsin, one part; absolute alcohol or rectified spirit, ten parts; carbolic acid solution (5 p. c.), ten parts. Leave the preparations in the watch-glass for twelve to twenty-four hours, unless time is an object. In the latter case heat the fluid gently until vapor is given off, then drop the films on the surface, and leave them for three to five minutes only. Next transfer the covers to an aqueous solution of sulphuric acid (25 p. c.), and when decolorization is complete, as evidenced by the pink coloration not returning when the specimens are plunged into a bowl of tap-water containing a single drop of ammonia solution, thoroughly rinse in the slightly alkaline water and counter-stain in an aqueous solution of methylene blue. Finally, wash in water, carefully dry and mount in Canada balsam. The bacilli should stand out as bright red rods on a blue background of cells, etc.—*Practitioner*.

## NEWS AND NOTES.

Culture media in test-tubes may be solidified either in an upright or a slanting position. The former is the one most useful for *stab* cultures, but the slanting position offers a larger and easier inoculating area and can be used for stab cultures if required. To inoculate a tube it should be held between the first finger and thumb of the left hand, with the slanting surface of the medium pointing upwards, and by keeping the tube as nearly horizontal as possible any germs in the air (which have a weight of their own) are prevented from falling into it. The infective matter, or second tube, should be held between the first and second fingers of the same hand, and, taking the needle high up the handle by the thumb and first two fingers of the right hand, sterilize it by placing in the flame till red hot, then with the little finger and palm, or with the backs of the ring and little fingers also of the right hand, remove the cotton-wool plug from the infective tube by a twisting movement. This screwing motion removes the cotton-wool fibres from the mouth of the tube more thoroughly than would be the case if the plug was drawn straight out. And now, as quickly and cleanly as possible, remove from the infective tube a *very little* of the specimen on the point of the needle. Plug the tube; remove the wool from the fresh one, and inoculate the new medium by making one, two, or three streaks the full length of the culture surface, and avoid touching the side of the tube. Withdraw the needle; pass the mouth of the test-tube two or three times through the flame, do the same with the plug-end of the cotton-wool, and insert it firmly in the tube. Sterilize the needle before laying down. Label the tube with date and hour of inoculating, and the source of the infective material, taking care not to wet the label with the tongue, as we are dealing with infective matter. Place in the incubator. In the case of *stab* cultures, the infective material is planted by thrusting the straight needle into the substance of the nutrient medium for its full length.—*Jour. State Med.*

---

An apparatus for making anaerobic cultivations in fluid media consists of a round-bottomed flask or stout bottle (A) fitted with a single-bored stopper, through which passes a right-angled bend of manometer tube (B). This is connected by thick-walled rubber tubing with another bend of glass tube (C), which passes through a cotton-wool plug into a test-tube or small flask (D); the rubber connection carries a screw or spring clip. The apparatus having been sterilized by moist heat, the bottle is nearly filled with the medium, about 15 c. c. of which are placed in the tube D; the whole is then sterilized for half an hour on two successive days in the steamer, the clip being left open, and the tube C being drawn up above the fluid in D. On the third day the bottle is placed in a calcium chloride bath (immersed up to its neck), which is maintained at a temperature of about 120° C., until all the air is driven out (five minutes will suffice to displace 50 c. c. of air); the tube C is then lowered into the medium, and the clip fastened. To inoculate the medium in the bottle a fluid culture or broth emulsion must be used; the tube C is removed from the test-tube and dipped into the culture; on opening the clip the infected fluid in the tube will be sucked into the bottle, and when a sufficient amount has been transferred the clip is again shut. The condition in the bottle is one of complete anaerobiosis, and the partial vacuum does not exercise any ill-effect upon the growth of the bacteria.—*Jour. State Med.*



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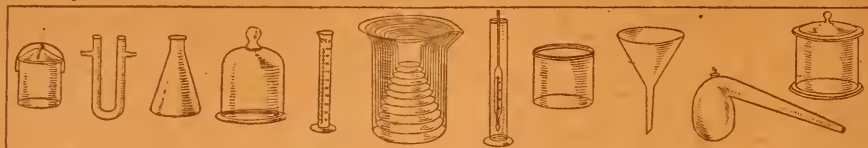
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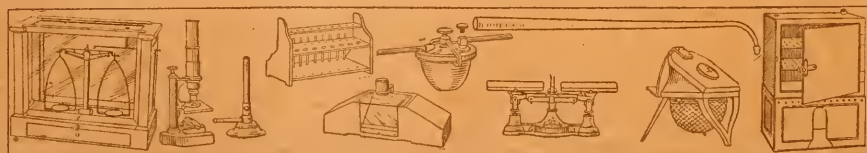
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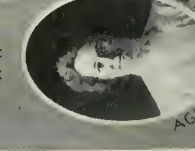
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# Journal of Applied Microscopy and Laboratory Methods

1902-3



# Journal of Applied Microscopy and Laboratory Methods

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SEPTEMBER, 1903.

NUMBER 9.

## New Apparatus for Phytopathological Work.

While working in the plant pathological laboratory of the University of Nebraska during the winter of 1902, we found it necessary to devise a number of pieces of apparatus to meet our peculiar needs. Of these, two have proved so generally useful as to warrant some published description. We wish to acknowledge our obligations to Dr. C. E. Bessey, under whose direction we were working, and also to two of our co-workers, Messrs. P. J. O'Gara and J. L. Sheldon, for assistance and criticism.

**A Transferring Oese.**—For isolating bacteria, the ordinary poured plate method is sufficient. For isolating fungi, and especially for obtaining cultures of fungi free from bacteria as well as other fungi, this method alone is ineffective, on account of the relatively slow growth of most fungi as compared with bacteria. Bacteria from a single colony may be transferred by merely touching the colony with a sterile oese and thus a pure and isolated culture be obtained; but the fungus plant or colony that has developed from a single spore must be transferred bodily, unless it produces spores with exceptional promptness. A single bacterial colony will often spread over the surface of a plate before the fungi in the plate have begun to produce spores. Thus it becomes desirable to either transfer the pure colonies (usually single plants) of the desired fungus to new culture dishes; or to remove the contaminating colonies of bacteria or other fungi bodily, leaving the desired colonies *in situ*. Moreover, in culture experiments involving growth for a month or more, it is often desirable to transfer a large colony to new or more moist medium; a simpler process than introducing new medium or water with precautions to insure sterility. At the beginning of our work we made such transfers with a two millimeter platinum loop; but this is not well adapted to the work; a small colony would slip through, a large one slip off, and the edge was not sharp enough to cut old and dried medium. Moreover, manipulation necessitated raising the cover of the culture dish so

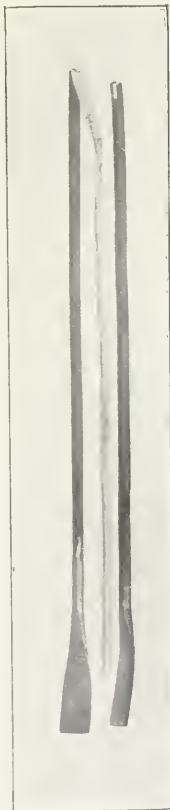


FIG. 1.

high and keeping it raised so long that there was danger of contamination. Apparently, however, there was no instrument on the market more suitable for doing the work. It was evident that something of the same general style as a section lifter was wanted, but with a blade and handle of a material to stand repeated sterilizing in flame; the blade must be firm, remain smooth after considerable use, and must be of the right curve and dip to slip smoothly under a colony, or, when inverted, to cut around it, without requiring the cover of the culture dish to be raised higher than the width of the handle; and it must be broad enough to pick up a large colony. After experimenting with models hammered from brass wire, a model was submitted to the Bausch & Lomb Optical

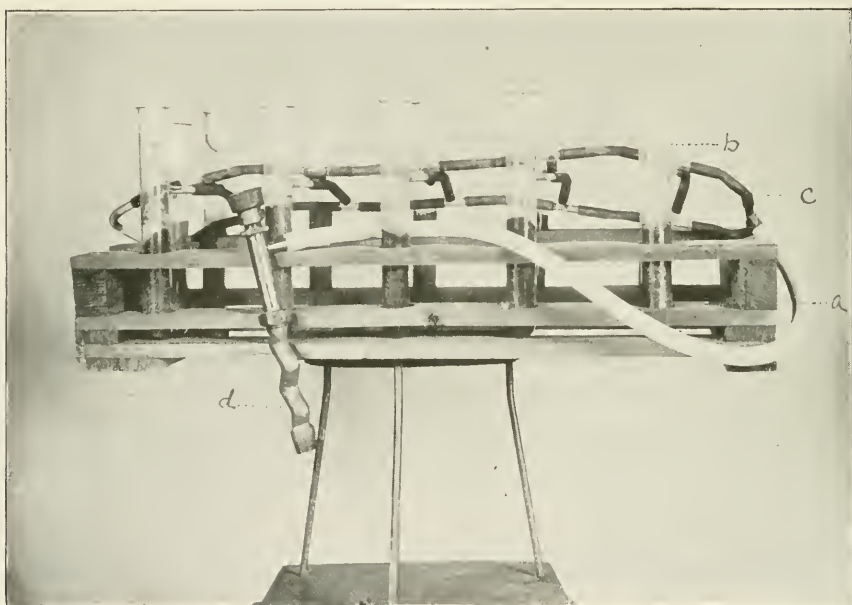


FIG. 2—*a*. Wooden frame, in which test tubes are set. *b*. Test tube, with side tube. *c*. Circular connection of rubber tubing, connected with side tubes by T-tubes. *d*. Richard's filter-pump.

Co., by whom the instruments similar to those shown in Fig. 1 were made. As completed these are of either steel or of copper, with the blade in two widths, one nine millimeters wide, the other four millimeters; length of blade, sixteen millimeters; length of handles, one hundred and thirty millimeters; making total length one hundred and forty-six millimeters. The narrow form is used less often than the wide, but is very necessary for certain work. It has proved more practicable to have the two separate instruments than to put two blades at opposite ends of the same handle; since the blades have almost a cutting edge. The instruments have now stood the test of over a year's almost daily use, and are still smooth and serviceable.

**An Apparatus for Growing Seedlings and Small Plants Under Sterile Conditions.**—In studying the phenomena of "damping off," it became necessary to devise



some simple method of germinating seeds and growing seedlings under absolutely sterile conditions. Large cylinders closed with cotton plugs were first used, but in these the seedlings did not grow well. As this appeared to be due to the complete lack of ventilation in the cylinders, various experiments were made to overcome this difficulty. The final result was the apparatus shown in Fig. 2. The unit of this scheme is a large test tube, to which a narrow side tube is joined about one-third of the length of the tube from the top—not a difficult piece of glass blowing. Damp earth is placed in the test tube; the test tube and side tube are plugged with cotton, and the whole sterilized fractionally in an Arnold sterilizer, or better, in an autoclav. The seeds to be used are then sterilized, and one or more introduced into the tube and planted at the proper depth, observing the same precautions as in inoculating a tube. After the seeds have germinated, or before if desired, the side tube is connected by rubber tubing with a filter pump. Air is thus drawn into the test tube through the cotton plug at the top, and out at the side tube, which thoroughly ventilates the growing plant. In practice we connect ten or twelve tubes with one filter pump, as shown in the figure. This is about the practical limit of number, since more air is drawn through the tubes nearest the filter pump than through those farther away. The air circulation necessarily dries out the soil slowly, which necessitates the occasional addition of water. This is done with sterile water and a sterile pipette in the usual way. We have each had apparatus of this kind in nearly continuous use for over a year, and have grown beet, cotton, rye, wheat, and pear seedlings without difficulty, inoculating them with pure cultures of various organisms associated with “damping off,” and with specific diseases. The most careful tests have shown that perfect sterility can be maintained in the tubes if they are carefully plugged and the usual precautions observed.

Clemson College, S. C.

Botanical Garden, St. Louis, Mo.

HAVEN METCALF.

GEORGE GRANT HEDGCOCK.

## A New Method of Sprouting Pollen Grains.

To obtain preparations of sprouting pollen for class use, it is usually the practice to make cultures in sugar solutions of different strengths, experiment being necessary to get the most favorable concentration for each kind of pollen. While this is the most common and in most cases the best method, it is not the only one that may be employed. Juranyi, for example, used the fresh cut surfaces of ripe pears and got satisfactory results with the pollen of *Ceratozamia*.

A method that I have used is to take advantage of nature's own medium for this purpose, i. e., the liquid secreted from the stigmas themselves. It is obviously not possible to make the method generally applicable, but when it can be used it is not only more interesting, but also more instructive to the students. The plant best fitted for this purpose, and one which is fortunately not hard to obtain, is the white Bermuda or Easter Lily so common with florists in the spring. The liquid secreted from the stigmas of this plant frequently collects into good sized drops that can be removed with a pipette. Make a hanging-drop culture

of the pollen of the lily in this liquid and there will be no difficulty in getting good long tubes for study.

I have not yet tried it, but it would be an interesting experiment to see if the pollen of other plants would grow in the stigmatic liquid of the lily. Results of this kind might be instructive when compared with experiments in hybridization and on the prepotency of pollen.

W. C. COKER.

University of North Carolina.

## Greenhouse for Pathological Investigation Under Control Conditions.

The Department of Botany and Vegetable Pathology of the Maryland Agricultural College has designed the greenhouse illustrated below, to meet the needs existing for the study, under control conditions, of injurious fungi or other plant growth. The greenhouse here illustrated from the constructor's plan is designed

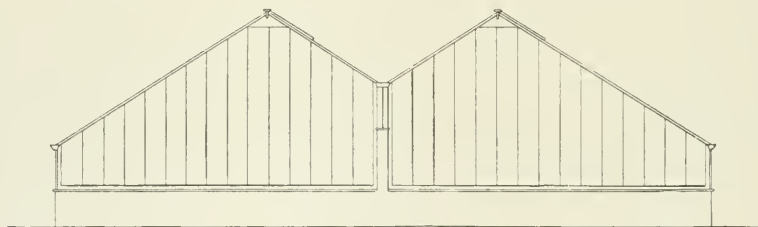


FIG. 1.—End view.

to give two main conditions, a warm and a cool room, and seven secondary conditions, each of which may be varied either five or ten degrees from the next lower compartment by the opening of valves controlling additional heat cells. The specifications call for sufficient valves, etc., to give the above control to each

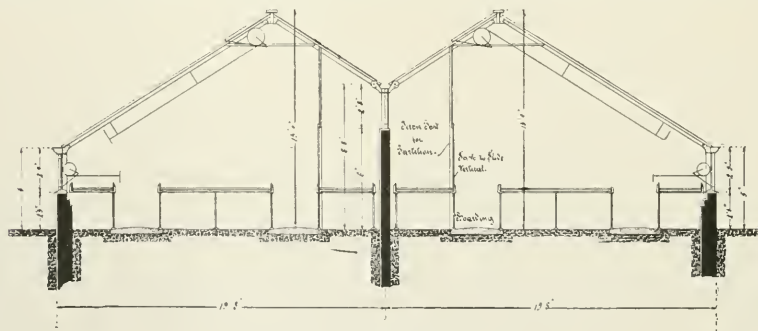


FIG. 2.—Elevation.

compartment and also to cut off either of the main sections of the house, any or all of them independently of the rest. The design was to obtain controlled conditions with temperatures, and humidity also, of different degrees, so that any question that might arise could be studied under the optimum as well as maxi-

mum growth conditions, and hence be able to advise treatment, especially in the case of fungi.

To meet a similar need in the Department of Entomology, it was designed to duplicate the first house, with all items, except in the position of the high wall bench. The plans as here shown give the ground and sectional views as prepared to meet the specifications prepared by the writer. Unfortunately

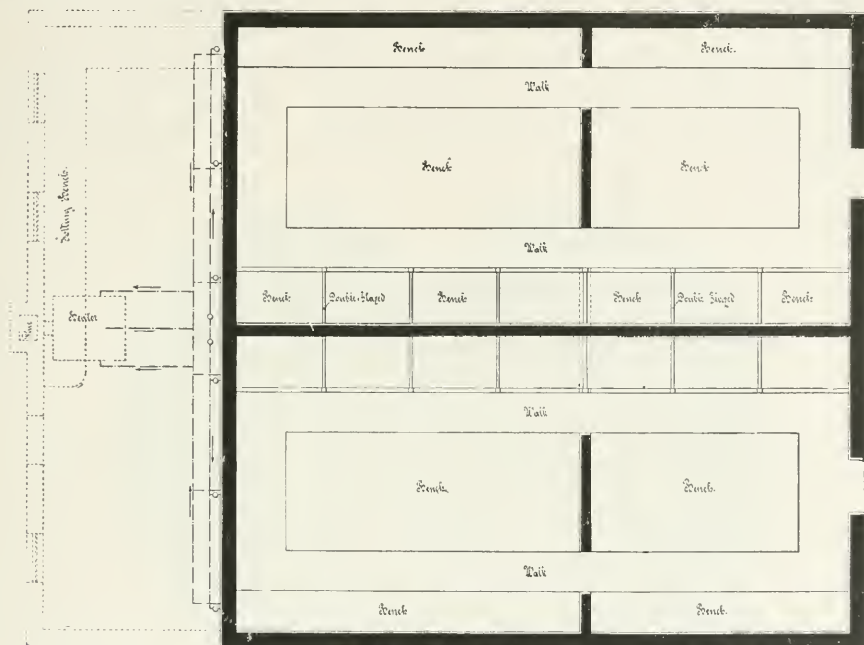


FIG. 3.—Ground plan.

the state legislature did not make any appropriation, at its last session, which could be used for the above purpose. Hence we have only the plans and experience derived by correspondence, to make use of when the opportunity arises. But as the house, as designed, includes some features not previously noticed elsewhere, and is in a sense a laboratory, it may be appropriately described in the JOURNAL.

FREDERICK H. BLODGETT.

Maryland Agricultural College.

Ruge (*Deutsche med. Wchnschr.*, Leipzig, 1903, No. 12) refers to Ross's recently published method of using thick layers of blood and staining with eosin (fifteen minutes) and methylene-blue (a few minutes), in greatly facilitating the demonstration of the presence of the malarial parasite. He strongly recommends it, and urges its superiority to the older method of thin films. He thinks it to be an improvement, however, to fix the film first. This he does by placing it in 1 per cent. formalin, then in  $\frac{1}{2}$  to 1 per cent. acetic acid, and then in 2 per cent. formalin. Elegant preparations are not given, but the parasites stand out prominently and are easily found, particularly crescents, which are often so sparingly found in thin films. He thinks that Ross's method is a great saving of time and trouble and is more sure.—*Edinburgh Medical Journal*.

## Sectioning of Wheat Kernels.

During some investigations being made by this bureau upon the chemical and structural characteristics of certain wheats, it became desirable to make sections of the whole grain, but the hardness of the kernels made it necessary to modify the ordinary methods of embedding. The method finally adopted consisted of softening with glycerine and then embedding in paraffin.

After making several sets of experiments to determine the effect upon the grains of various percentages of water and alcohol, and of water and glycerine, it was found that not more than from 5 to 10 per cent. of water should be present.



FIG. 1.—Photo-micrograph of cross section of wheat kernel.

In detail the method is as follows: The grains are first soaked in 90 to 95 per cent. alcohol for several days (10 to 14), after which 90 per cent. glycerine is added to the alcohol in small portions at intervals of a few days until the proportions of alcohol and glycerine are about equal. The material is then allowed to stand until the grains have attained a firm, cheesy consistency. This time will vary with the nature of the grains from three weeks to as many months. A larger per cent. of water will hasten the process, but the grains will be swollen by it. When the softening has been completed, a layer of the material is exposed to the air in a shallow dish with a sufficient quantity of the liquid to cover it. In two or three days the alcohol will have evaporated, thus leaving the grains infiltrated with the glycerine and a very little water. To embed, the following



procedure was found to give good results, since it allows the paraffin to penetrate only the seed coats and prevents the removal of the glycerine :

1. Alcohol (98 per cent.), 30 to 60 minutes.
2. Chloroform, 30 to 60 minutes.
3. Chloroform with paraffin shavings, 60 to 90 minutes.
4. Place in paraffin oven with melted paraffin 2 hours, changing the paraffin two or three times.
5. Make into a cake as usual.

Complete sections of a thickness of 15 to 20  $\mu$  can be cut from material prepared by the above method. The comparatively large size of the starch grains seriously interferes with obtaining good sections of the endosperm much thinner than the above. The sections can be fixed to the slides with albumin fixative as usual. After removing the paraffin it was found best to use alcoholic stains. Kleinenberg's hæmatoxylin followed by alcoholic eosin and then followed by absolute alcohol, xylol and xylol balsam makes a good combination.

Bureau of Chemistry, U. S. Department of Agriculture.

BURTON J. HOWARD.

## A Useful Modification of the Life Box.

Professor Osborn's article "On the Use of Compression in the Study of Small Organisms," in the July number of the JOURNAL, has suggested calling the attention of biologists to a little device which I have employed for a number of years, though I do not remember to have seen it described nor to have known of its being used by others. It is a very simple modification of the life box (B. & L. No. 4785) which greatly increases its usefulness by enabling the observer to surround the object under observation at any time with any desired reagent. It consists merely in substituting in place of the ordinary cover-glass of the life box a thin glass perforated by a small opening near one margin (Fig. 1). As cover-glasses of the ordinary thicknesses are too fragile to stand being bored, I use a cover-glass from  $\frac{1}{2}$  to  $\frac{3}{4}$  mm. thick cut from a sheet



FIG. 1.

of glass such as is made for mounting brain sections. This thicker glass is not only strong enough to stand boring but has for this use several other advantages over the ordinary thin cover-glasses furnished with the life box. It is not deflected from its position by pressure from below and the object beneath is therefore held between two parallel *plane* surfaces, thereby obviating the sliding of the object or the movement of the fluid about it from one part of the box to another where the pressure is less or where surface tension is greater, as happens frequently where a thinner flexible cover is used. Moreover, the amount of pressure put upon the object is controlled directly by the observer instead of depending on the elasticity of the cover-glass. A cover-glass of this thickness allows the use of medium powers (I use Zeiss D) giving ample room to focus as deep as necessary. The essential feature is, however, the opening in the cover through which any fluid may be introduced beneath the glass

without in any way disturbing the position of the object. The hole is about one mm. in diameter and 5 or 6 mm. from the edge, thus being above the marginal portion of the raised dais which forms the floor of the box. Fluid introduced through the hole is therefore held by surface tension between the two glasses which form the top and bottom of the box, and so is prevented from contact with the metal parts. The use of corrosive reagents (corrosive sublimate, e. g.) is not, therefore, prevented.

In fixing highly contractile organisms, such as trematodes, for permanent preparations the life box as thus modified is very useful. Such animals usually contract strongly, taking on various unnatural positions immediately upon coming in contact with fixing fluid. But a normal position may readily be gotten by putting the animal in the life box in a small drop of water, normal salt solution or other non-irritating medium, and deliberately adjusting the cover until the exact position and degree of compression desired are gotten. Then (not before) the fixing fluid is run in around the animal by means of a small pipette and allowed to act as long as necessary to produce fixation in the position in which the animal is held. When fixation is completed, or, if preferred, as soon as all power of contraction is destroyed, the cover is removed and the animal transferred to a larger body of fixing fluid, or to such other reagent as may be desired.

The aperture in the cover-glass in no way interferes with the use of the life box for the long continued observation of living organisms, but gives the additional advantage of allowing any desirable position or condition to be made permanent at will. I know of no other means by which the same result may be so satisfactorily accomplished.

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## The Technique of Biological Projection and Anesthesia of Animals.

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### XVII. DIRECTIONS FOR MOUNTING LIVE ORGANISMS FOR PROJECTION.

These directions are especially applicable to mounts made for use on projection microscopes of the direct type, as illustrated in articles III and V of this series (vol. V, No. 5, Fig. 2, and No. 7, Fig. 4), in which the stage of the microscope is in the vertical plane and the pencil of light rays is not reflected by a mirror. Projection microscopes with vertical reflectors offer the one advantage—desirable in a small percentage of all cases—of having the stage of the microscope in the horizontal plane; but this advantage is offset by loss of light, difficulty of adjustment, and additional expense. All the forms of cells shown in Fig. 9, except Nos. 14, 16–20, which have open tops, may be used on horizontal stages and the organisms are mounted in them as described below for use on the direct type.

In selecting the size and shape of cell to contain an organism which is to be

projected, the purpose of the experiment must be considered as well as the form and size of the organism, especially when the specimen is an active animal or one of the interesting motile plants. It may be desired to exhibit either the general morphology and characteristic motions of the organism, which are, in general, best seen under a relatively low power objective, or anatomical details and functional activity of organs, which require a higher magnification. For example, one may desire, at one time, to exhibit the movements, changes of shape, somites, and setæ of an earthworm; and again, to demonstrate the animal's thin-walled crop, muscular gizzard with contained food and sand grains, the pulsating dorsal vessel and hearts, and the nephridia. In the first case, a comparatively large cell, Fig. 9, No. 11, and a large worm, or two, are used; while, in the second case, a compressor, No. 15, or a life box, No. 10, and a small transparent bodied worm under the influence of chloretone will give the best results. The low power objective will have a much longer working distance and may be used, therefore, with a deeper cell than is required by the short working distance of the higher power lens. In medium and high power projection, therefore, shallow cells are absolutely necessary and are desirable, even when using low powers, because in them moving animals are more easily kept in focus.

Cells with sloping sides, e. g., Fig. 9, Nos. 3, 7, and 13, are, in general, better than those having straight sides as in Nos. 12, 17, 18, and 21, since the animals can not find a hiding place close against the side of the cell, nor is there any interference with the passage of light through the cell. Thin bodied animals, e. g., planarian worms, leeches, and small larvæ, should not be mounted in cells with straight sides, which are better adapted to thick bodied, active species and to non-motile species, e. g., *spongilla*, bryozoa, and macroscopic plants, as in the evolution of oxygen.

Very small organisms, ranging in size from bacteria to small water fleas, need only the form of mount adapted to high power objectives.

*A. Directions for mounting specimens of microscopic size, such as amœbæ, infusoria, bacteria, desmids, diatoms, yeast, oscillaria, spirogyra, vaucheria and similar forms.*

These types are to be mounted in essentially the same manner as for ordinary microscopical examination, only slight modifications being necessary to adapt the method to the requirements of a projection microscope. Place the organisms in a drop or two of clean hydrant water, or water in which they have been living, on a clean polished plate glass slide, or, if this is not at hand, on a slide selected for clearness and flatness. Polished plate glass slides are preferable because their flatness and even thickness requires re-focusing less frequently while the object is being moved about under high power objectives. Over the objects place a three-fourths or seven-eighths inch thin, clean, selected cover-glass, being careful to exclude air bubbles. Large cover-glasses hold more water and prevent rapid destruction of the mount by drying. If there is just enough water on the slide to fill the thin space between it and the cover-glass, the mount may be turned up on its side without displacement of the cover; but if there is too much water, the cover will float off when the slide is tilted. To

remove any excess of water either absorb it with clean filter or white blotting paper, which should be placed on the slide in contact with the water, *but not upon the cover*, and as the water is absorbed tilt the slide so that the excess of water will flow around the cover to the absorbent paper. Or, as small pipettes, or medicine droppers, with rubber bulbs are, or ought always to be, on the working table, they may be used as successfully and quickly as absorbent paper. Place the point of a fine pipette in the water at the edge of the cover-glass and tilt the slide slowly toward the pipette as the water is being drawn up.

If the objects to be mounted are so thick and delicate as to be crushed between the slide and cover by the pressure of the latter, the cover is to be supported by a bit of thread, broken cover-glass, or paper.

If the specimen dries out to such an extent that more water is needed, it is not necessary to remove the slide from the stage. With a pipette place a *small* drop of water near the edge of the cover and in contact with the water under the cover, and it will run in. Chemical tests, e. g., the iodine test for starch, may be applied in this way, so that an entire class may see the inflowing chemical and the reaction it produces.

Actively motile diatoms may be seen to move in all directions, including vertically upward against the force of gravity, when mounted and projected as here described. *Oscillaria* and *volvox globator* exhibit their characteristic motions.

*Special method for mounting paramæcium and similar active and free swimming species of microscopic size.*

While chloretone, as indicated in articles VIII to XIV, offers the best method for controlling the voluntary activities of most species of animals during laboratory study and projection experiments, certain species of infusoria, notably *paramæcium*, offer a peculiar resistance, the cause of which has not been fully investigated, to its successful use. The following method of trapping these minute forms in spaces not larger than the diameter of the field of the objective used in their study was devised by the writer and has worked well in ordinary class work and on the projection microscope. A small piece of clean Japanese lens paper is pulled carefully by its edges until it has two to four times its original diameter, thus separating its fine translucent fibers into a network of almost cob-web-like delicacy. Place a piece of this about the size of the cover-glass on the center of a clean plate glass or selected slide and drop upon it the water containing the organisms, and cover with a cover-glass, which is to be pressed down very gently and the excess of water removed, as above directed. The animals will be found entangled in the meshes or caught under the fibers in such ways that their motions, changes in shape as they pass between obstacles, and their structure may be readily studied.

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University of Chicago.



## The Museum.

### X.

#### THE CASE.—Continued.

##### PIER WALL CASES.

The T cases previously discussed are good illustrations of pier cases, but as they may be regarded as anomalous, the ordinary box shaped examples are here considered. The pier case extends out into the hallway, usually from the walls, between windows. It is obvious that its dimensions can be much varied, dependent on the purposes it is to meet and the space, between windows, it is to occupy. Such a space should not be excessive. It should hardly exceed nine feet for all cases holding shelving and intended for small objects. In many instances where the wall space is greater and wide cases can be built they may be made into group cases which do not demand extreme illumination for their inspection. Pier cases can be carried outward from the walls into the hallways until the hall becomes a succession of alcoves or rooms with a corridor between. Or *vice versa* a central partition frame may divide the hall into two halves, and



FIG. 47.—Wall cases, broad and narrow, between windows.

against this the pier cases can abut, extending out towards the windows. It is clear that in such a disposition both sides of the hall must be provided with windows, and preferably as many windows as alcoves, and opposite to them. The extreme length of the alcove cases is a disagreeable feature, the hall effect is obliterated, and the table cases, so invaluable for many objects, are expelled. As has often been remarked, it serves the purpose of breaking up the hall into a number of compartments which can be individualized by some special contents, and so the series of alcoves become involved in a developmental or pedagogic system. Generally speaking pier cases may extend from the wall, in a hall 60 feet wide, 18 feet. Pier cases should be usually divided by a partition through

the center. Such a partition can be carried from end to end, or at the free or distal end cross shelving can be introduced, and may be of wood or painted canvas.



FIG. 48.—Pier wall cases of identical size. Bird gallery, New York Museum.

The varying forms, dimensions, and especially window spacing permit or exact corresponding variations in the disposition and size of pier cases. Figure 47 shows the pier broad and narrower and adapted for groups in the Mammalian exhibits of the New York Museum on a gallery floor; Figure 48, the pier cases



FIG. 49.—Narrow pier wall cases. Peruvian Hall, New York Museum.

of average identity in form and size in the Bird gallery of the north wing of the same museum; and Fig. 49, the pier and interposed cases (free from the walls and opposite the division frame of a double window), used serviceably in the Peruvian hall of the same institution. In the first the broader cases are eight feet in width, and the narrower four, while all alike, are about nine feet ten

inches in height, and nine feet in extension from the wall. In the second the cases are seven feet long, four feet wide, eight and a half feet high. In the third the broader cases are three feet in width, ten feet in length, eight feet four inches in height; the narrower, two feet in width, eight feet in length, eight feet four inches in height. The partition, shelving, and appearance of exhibits are indicated in the photographs.

In Fig. 50 some working details are depicted (not in scale) of a pier or pavilion case without partition or diaphragm, for which I am indebted to A. B. Strader; *a* being the construction of the wall of the case with sashes, *b* the construction of the cornice, *c* that of the base. Pier cases, as shown in Fig. 49, are frequently double-front cases or rectangular constructions, detached from the walls, with a diaphragm or back passing from end to end through the center. They are two wall cases back to back. Such cases are useful and might advantageously replace all wall-cases where wall-cases cannot be so favorably built as to receive the light from the windows.

These double-front cases should be on heavy iron wheels or rollers, hidden by a marginal skirt of wood or stone. And, indeed, all cases, where it is feasi-

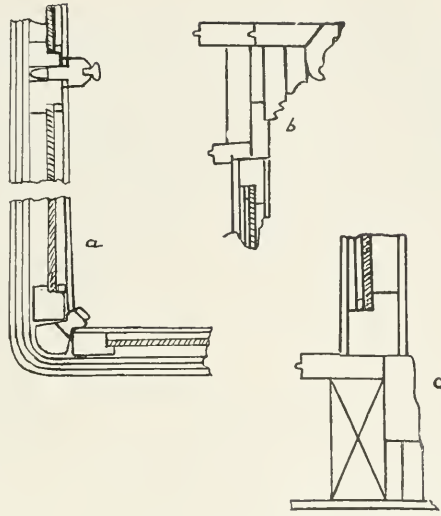


FIG. 50.—Working details of a pier case.

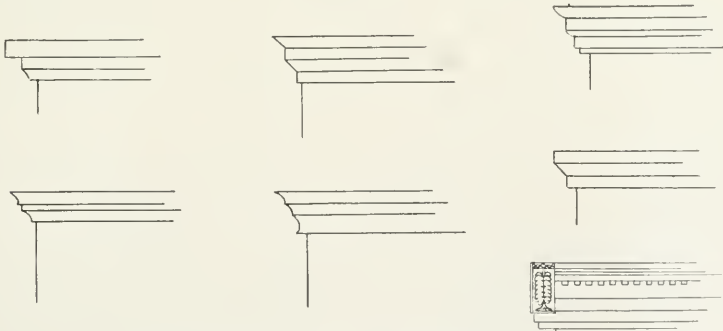


FIG. 51.—Commendable forms of moulding cornices on wall cases.

ble, should be movable. I have seen the most unfortunate strains given to cases and the most unlucky injuries inflicted on men by the hardship of having them *shoveled* into new positions.

A feature of some importance in wall cases of all kinds is the cornice. A great many complicated and heavy designs may be resorted to, but it seems obvious to good taste and judgment that the more simple are to be preferred. At any rate, whatever combinations of fillet, ogive, or scroll are adopted it

should be insisted that all resting surfaces, where dust might lodge, should be *faced to the floor*. This is not an unimportant consideration; the dust covered tops of *upturned*, instead of reversed, cornices, on high cases, is a distinct disfigurement to a museum, and involves incessant attention in the matter of cleaning. Again, the removal of dust from high positions is usually accomplished by the use of a pole duster, and the consequent cloud of dust subjects all the parts of an exhibit, in and out of cases, to a fresh invasion, every time the cornices are

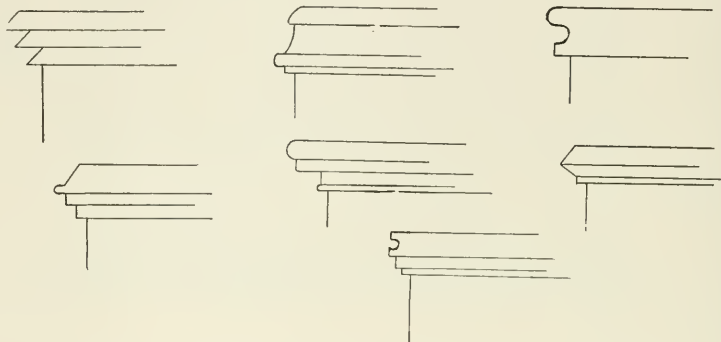


FIG. 52.—Objectionable forms of moulding cornices on wall cases.

cleaned, of this defacing and omnipresent museum misery. Figures 51 and 52, suggestive simply, illustrate correct and incorrect systems of cornice design.

The shelving of cases may be here considered. The shelving in cases has two objects, provision of room for the specimens exhibited, and favorable positions for their intelligent examination. The second may be considered paramount, except in such instances, where the exhibition cases become storehouses. Probably the best system of shelving involves placing the widest shelf the first

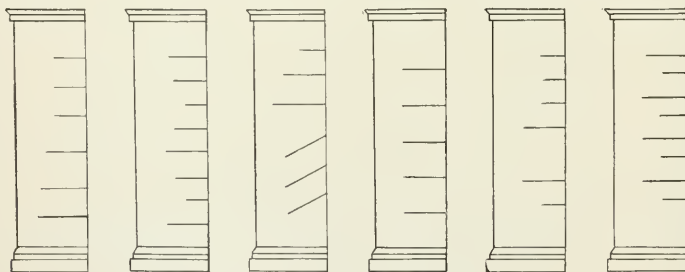


FIG. 53.—Types of shelving.

above the bottom of the case, at about two and one-half feet below the level of the eye of the spectator and carrying the shelves above that in gradually receding order, so that the narrower are at the top. The smaller objects, with some admixture of larger ones, for the effectiveness of its appearance, should be placed on the lower shelves, the larger above, except such large objects as can be accommodated on the floor of the case.

The shadow of the upper shelves, by the method of progressive shrinkage in width, is less apt to obscure the objects below. Shelves can be slanted by put-



ting sloping blocks on the brackets, or they can be more steeply inclined by carrying up the shelf until its front edge falls within the pin of the bracket, and rests on that, inasmuch as the pin which fits into a socket or hole on the underside of the shelf lies from two to four inches behind the front edge of the shelf. This pin can be concealed in many ways, readily suggested by inspection. With large objects the shelves may be tiered directly one above the other of equal width. Besides tilting in the two ways suggested above, sloped brackets are made which give a less but useful inclination. Shelves brought close to the front of the case exhibit objects nearer the eye of the visitor, and hence for objects of a uniform size, as cubes of building stones, wall cases of the requisite depth with shelves of one width from top to bottom, directly superimposed at equal distances, serve an admirable purpose. The shallow

and *appliqué* effect of such cases is sometimes disappointing. The case loses atmosphere, and the receding shelves in deeper cases from the bottom upward produces a pleasant impression. The *bay* arrangement of shelving is to alternately widen and narrow the shelving. Figure 53 presents these modes of shelving, with others.

The support of shelves in high cases seems adequately secured in three ways: (1) by the arm-bracket (Fig. 54), and (2) the bayonet bracket (Fig. 55), and (3) small bracket (Fig.

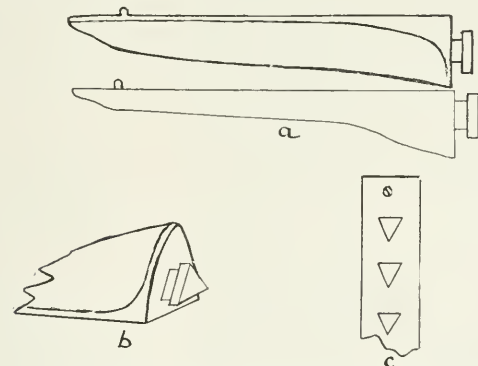


FIG. 55.—Bayonet Bracket showing ratchet, proximal end, and reinforced bracket.

56), the two latter fitting upon perforated ratchet strips, the former upon a ratchet strip with iron teeth. In the arm bracket, the brackets vary of course in length according to the dimensions of the shelf to be supported, and as the bracket lengthens the subordinate *arm* underneath moves further and further out under the bracket, and at the same time diverges, below, from it more and more, thus keeping the angle of strain unchanged (see Fig. 54). A very satisfactory relation is obtained by making the point of attachment of the lower arm one-half (or the middle) the length of the bracket, and its divergence from the bracket one-fourth of the length of the latter. The teeth on the ratchet strip are one and one-quarter inch apart. These brackets are cast iron, are serviceable in all cases, and especially are suited for the support of heavy weights.

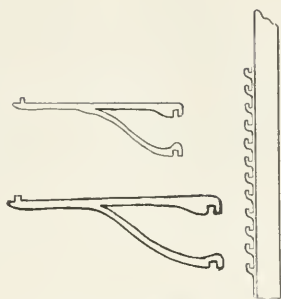


FIG. 54.—Arm bracket with ratchet.

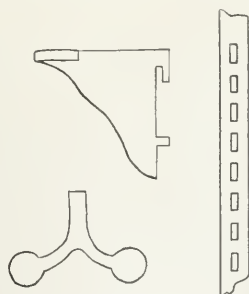


FIG. 56.—Short bracket, showing ratchet and support.

The bayonet and short bracket are perhaps more pleasing in appearance, are not so obvious, but are only safely used for less heavy objects. With glass shelves the short bracket produces excellent results, and for pottery, glass, small figurines, etc., seems admirable. For greater strains the bayonet bracket can be reinforced by thickened and deepened keel (see Fig. 55).

The use of glass shelves in high cases has received a great deal of endorsement, and certainly for many types of exhibits can be favorably regarded. Glass shelves, as being unpainted, remain permanent in appearance, and can be cleaned far more perfectly and easily than wood; they are quite strong, and their elusiveness and transparency to the eye gives the interior of cases a more agreeable expression than wood, in many instances. On the other hand, wood is strong, and for minerals, fossils, economic exhibits, building stones, etc., even for some zoölogical exhibits, is to be preferred, while wood, as being adapted to the use of colors, can be conveniently used as a background for the heightening of effects.



FIG. 57.—Wall cases covering angle between walls. Crane Museum, Pittsfield.

And of course, in the exigences of museum installation, when shelves must be sawn in two, or apertures and openings made through, to accommodate high or irregular specimens, wood has a plasticity quite irreconcilable with the use of glass. Iron shelves painted or covered with leather can hardly be recommended. They are incombustible, but they are very heavy and clumsy, and offer but few advantages.

The interior walls of high cases can be made of wood or lined with zinc, to insure dryness, or a board used covered with a linoleum cloth. This latter device furnishes an attractively grained background, is not subject to the warping and folding of metal plates, and is not impervious to tacks, nails and other fixtures.

A word may be profitably added upon the inexpediency of putting wall cases past the angles or *corners* of halls. A wall case built upon two walls and covering also the angle between them (speaking only of rectangular halls) while in a

small room it may be pleasing, offers poor exhibition space at the angle of the case, and for reasons of construction and general use is injudicious. Figure 57 shows such a wall case covering the angle between two walls in the Crane Museum at Pittsfield.

The flat or table case is the indispensable adjunct of museum installation. It comprises the various designs of cases intended for floor use where smaller or flat objects are exhibited, and it meets the wide range of needs in libraries, art museums and schools, where groups, figure pieces and tall and upright objects are less frequently seen.

There are two sorts of table case, the desk, single or double, and the inverted V (or A) case on legs. Desk cases of great beauty have been prepared in num-



FIG. 58.—Table case, showing drawers and cupboard beneath.

bers, in the New York Museum, and it would be difficult to suggest anything more propitious for its objects than these (Fig. 58). They are made usually with three sashes, giving them a length of some eighteen feet, and have two sloping sides—one inch in seven—the double desk. They are fitted with green plush on the bearings of the lids, as a dust prevention. Some of these cases are a trifle broad, and the objects at the back of the case are indifferently seen. The best dimensions, using the lettering in Fig. 59, are as follows: a-b, 5 ft.; b-c, 8 in.; d-e, 12 ft.; d-f, 2½ ft.; f-g, 3 ft. 2 in.; g-h, 4 ft. 4 in.; g-a, 2 ft. 5 in. Details of construction are given in Fig. 60, for which I am indebted to Mr. A. R. Strader.

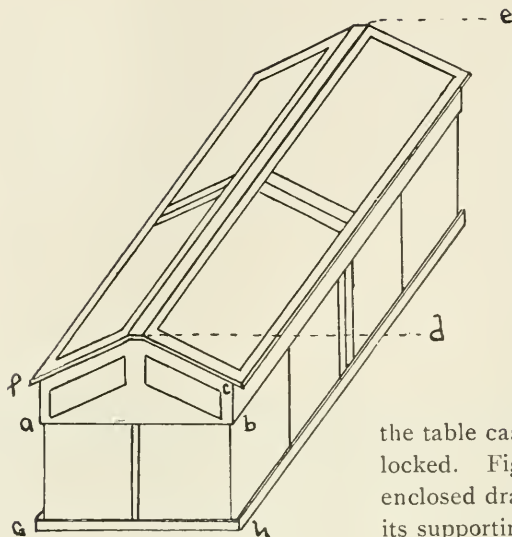


FIG. 59.

The drawers under the flat cases, as a convenience to curators, are invaluable. The artistic effect of these "bodies" is certainly unfortunate. The desk cases on legs forming no interruption to the untrammelled view of the floor of the hall conduce to the effect of size, and are distinctly more elegant. Figure 62 of the Hall of Vertebrate Palæontology in the New York Museum illustrates pleasingly the improved appearance of a hall furnished with cases on legs.

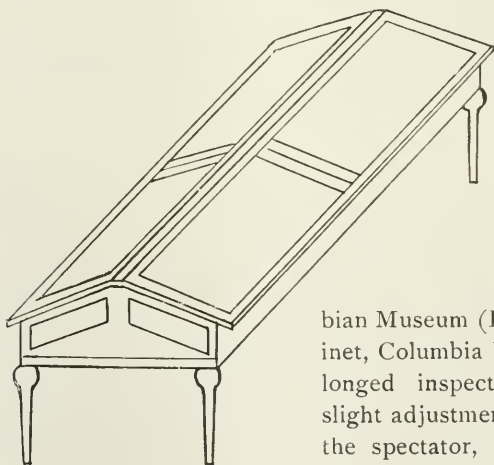


FIG. 61.

These dimensions, of course, may be changed indefinitely by slight alterations, but the example given will meet all requirements. Such flat cases can be raised on legs (Fig. 61), or they can be put on bodies or stacks of drawers which are to be used for putting away duplicate or unnecessary or overflow material, or specimens unfitted for public exhibition. These drawers are enclosed by double doors, in each section of the table case, which shut upon them and are locked. Figure 58 shows the open doors, the enclosed drawers, and the uplifted sash with its supporting arms.

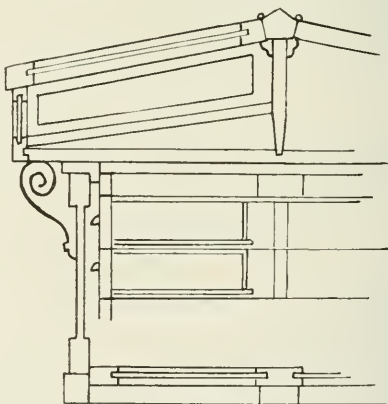


FIG. 60.

The flat or desk case, generally, in its larger or longer development, should have sloping sides. The ease of attentively regarding objects seems sensibly helped by this, whereas exhibitions in level topped cases, as in the Mining and Metallurgical Collections, Field Columbian Museum (Fig. 63), and the Mineralogical cabinet, Columbia University, grow tiresome after prolonged inspection. In museum equipment any slight adjustment that will enhance the comfort of the spectator, and prolong his attention, is to be carefully adopted, even when quite incommensurate





FIG. 62.—Hall of Vertebrate Paleontology, New York Museum.

in itself with the results it attains. The flat or desk case is sometimes modified by an upright addition in which larger objects can be placed, and which may serve to relieve the depressed look—squattiness—of the desk cases themselves (Fig. 64).

In museums of natural history this is really seldom successful. They cannot always be used appropriately, and unless the objects are large they serve no useful purpose. They are preferably replaced by a long, narrow box, divided by a partition and opening on top by lids, and supported on metallic standards (Fig. 65). In these receptacles, photographs, maps, sections, flat plans, dissections, labels, etc., can be placed, and made illustrative or explanatory of the exhibit in the flat case below.

In art museums this top case has more value; or in archæological and ethnological sections of natural history museums, where vases, pottery, folios, even



FIG. 63.—Mining and Metallurgical Collection, Field Columbian Museum.

objects of personal adornment, on paper backgrounds, can be displayed, they are available; though usually, if there is room enough in wall cases or special shallow cases (see below), it is best to abandon their erection.

The inverted V, or A, case is a useful and sometimes attractive form of case (Fig. 66). It can be filled within by a core or mat. This core takes the shape of a smooth or stepped pyramid. If the latter, the series of steps form shelves, upon which the objects are placed, ascending upward. If smooth, the core can be covered with baize, cloth, plush, etc., and the objects in some way attached. The white plaster cells holding lepidoptera are in this way arranged rather strikingly in such cases in the New York Museum (Fig. 67), by pins holding up the white blocks. Or these A cases may be fitted with shelves, and be adapted to various uses, inserting short brackets (see above) and glass shelves. Again, these A cases may be used for skeletons, animals, vases, etc., without cases. In

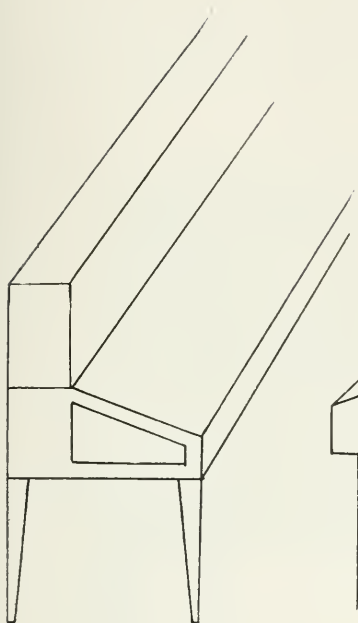


FIG. 64.

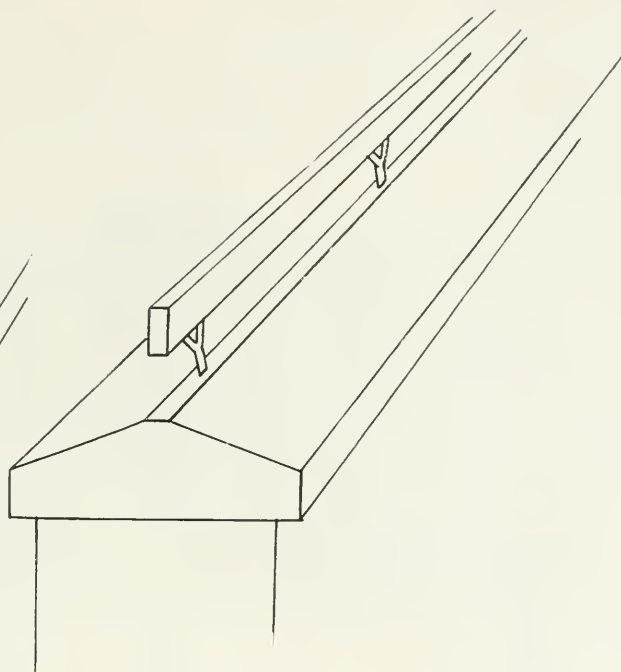


FIG. 65.

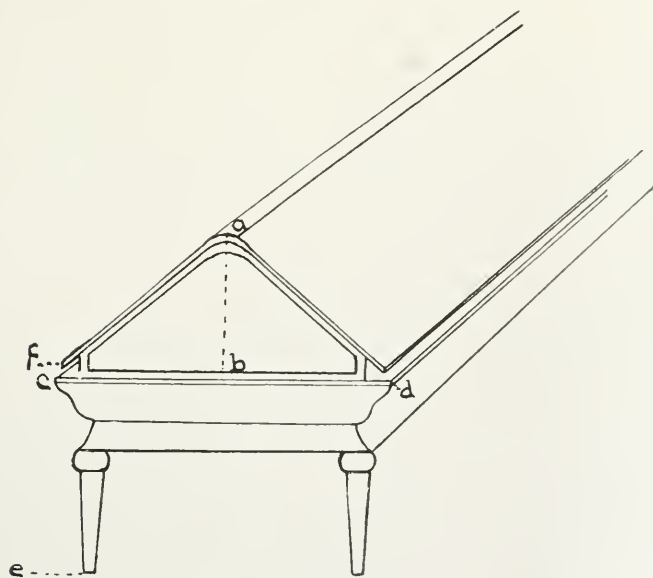


FIG. 66.

this latter case the effect is poor, and the case is evidently constrained to a purpose for which it is unadapted.

Very critically considered the A case does not appear to the writer desirable, as all the purposes it subserves can be more legitimately secured by some type of a hollow parallelepipedon or *cell*, the ultimate and artistic structural unit of the museum.

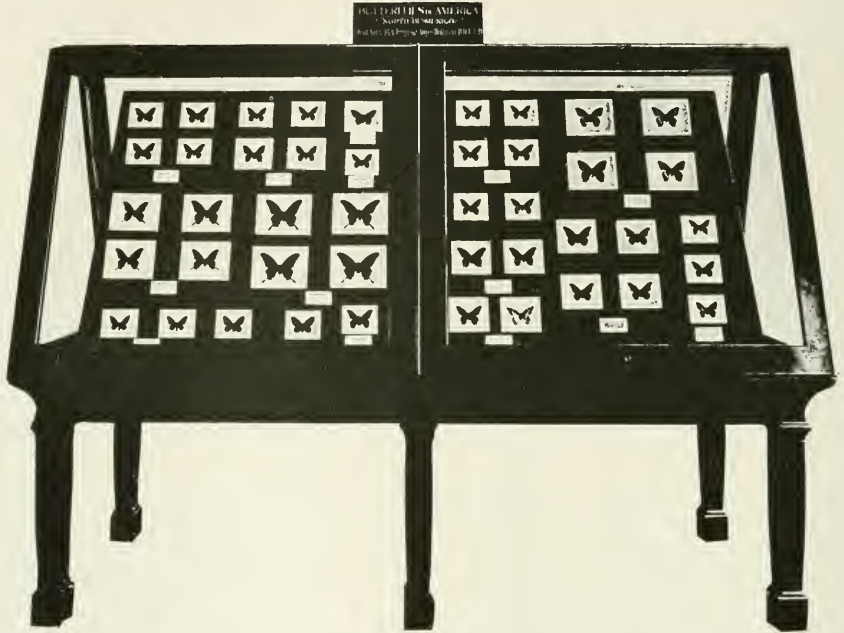


FIG. 67.—Specimen Case—Butterflies in A case.

The A cases are constructed of a metal frame, stiffening a wooden sheath, the doors on the side may be one (the whole length) or two (half-length), they open upward, are hinged at the top, and in the top a "light" is inserted. Dimensions for a typical case are as follows (compare Fig. 66): a-b, 2 ft. 9 in.; c-d, 3 ft. 4 in.; c-e, 2½ ft.; f-a, 3 ft. Length, eight feet, eleven inches.

American Museum of Natural History.

L. P. GRATACAP.

The method adopted by Dorset in using eggs as a medium for the cultivation of the tubercle bacilli is to mix together the white and the yolk in test-tubes, and then, by keeping the material in the tubes at 70° C. for four hours upon two successive days, it is both sterilized and coagulated. The use of the yolk alone does not produce a very abundant growth of the tubercle bacilli, and the use of the white alone is quite unsatisfactory. A couple of drops of sterilized water is placed in each of the tubes to moisten the material, and then the tuberculous matter is inoculated upon the surface of the medium. An abundant and satisfactory growth has been obtained from tuberculous material from guinea-pigs.—*Journal of State Medicine*.



## Methods in Plant Physiology.

## XV.

## RESPONSE TO CHANGES OF TEMPERATURE.

Select a tulip which is ready to open, or has very recently opened, and fasten it in a test tube or a small bottle of water by passing the stem through a hole in the cork. If the tulip is closed, place it in a temperature of  $20^{\circ}$  to  $27^{\circ}\text{C}$ . When it is well opened, transfer it to a temperature of  $8^{\circ}$  to  $12^{\circ}\text{C}$ . The change from warm to cold and from cold to warm temperature may be made several times, the temperature and the time being noted for each response.

## RESPONSE TO CONTACT STIMULUS.

1. **Experiments with Blossoms of *Berberis vulgaris*.** Explore with a bristle the blossoms of the barberry (*Berberis vulgaris*) growing in the open to determine in what stage of development of the flower the stamens are irritable. When this has been determined, pick and place in a damp chamber several clusters of blossoms and carry them to the laboratory.

While the flowers are still in the damp chamber, explore all the organs with a bristle to determine which ones and what parts are sensitive, dissecting off any organs that may interfere with the search. After dissecting off any members the blossom should be allowed to remain at rest for ten or fifteen minutes to recover from the shock.

When the sensitive area has been carefully localized determine :

(a) The latent period at room temperature, recording the temperature and having the water containing the blossoms at the exact room temperature.

(b) The latent period at  $8^{\circ}\text{C}$ .

(c) The time, at a given temperature, required for the return of an organ to its original position.

(d) The number of times, at a given temperature, an organ will respond to successive stimulation.

(e) The effect of an induced electric current. For this test the flower stalk should be fastened to one wire, and the other wire brought into contact with various parts of the blossom.

(f) The effect of an anæsthetic on the irritability. To carry out this test a few flowers are to be floated on water in three or four watch crystals, and one or more stamens of each tested for irritability. The blossoms that are found irritable are then to be placed, while still in the watch crystals, under a bell-jar having a capacity of 1 or 2 liters. At the same time a watch crystal containing about 1 c.c. of chloroform is introduced under the bell-jar. After five minutes have elapsed, the bell-jar is raised enough to allow the removal of one watch crystal and the sensitiveness of the stamens is tested. At the end of another period of five minutes another watch crystal is removed and the flowers tested, and so on until the stamens no longer respond to contact. Such insensitive blossoms are then allowed to stand in the open air and are tested at intervals of five minutes for the return of sensitiveness.

(*g*) The effect of an anæsthetic on the ability of a contracted organ to regain its position of rest. The stamens of a flower floating on water are to be caused to contract, and the preparation immediately placed under the bell-jar containing chloroform vapor. The subsequent behavior of the flower is to be observed.

2. **Experiments with the Sensitive Plant, *Mimosa pudica*.** In the following experiments use small potted plants and work in bright light.

(*a*) When the leaves of the plant are all well expanded, the pot may be gently raised and then set down upon the table with a heavy jar. The behavior of the plant is to be closely watched to determine the differing action of the various parts of the leaf. If the action was not followed for each part, the plant may be allowed to regain its expanded condition, and the jarring repeated.

(*b*) Beginning again with an expanded plant, the various parts of the leaf may be explored with a bristle for sensitiveness. By this means a careful record can be made of the most sensitive part of the leaf, and of the response following stimulation in various places.

(*c*) Allow the plant to regain its fully expanded condition, or take a fresh plant, and determine the latent period at a definite temperature. Allow the plant to regain its expanded condition. Stimulate again at the same temperature and in the same way, and determine again the latent period. Continue to repeat the test till the plant shows a great change in its response.

(*d*) Place a plant under a bell-jar and when it is fully expanded introduce a watch glass containing ammonium hydrate. If the plant gives any response, remove the bell-jar quickly to save the life of the plant.

(*e*) After causing the leaves of a plant to contract, cover it with a bell-jar under which there is also a small dish of chloroform. Note the subsequent behavior of the plant.

3. **Experiments with the Leaves of *Oxalis*.** These experiments should be performed in the sunlight.

(*a*) A potted oxalis with the leaflets of a leaf all lying in one plane is to be selected. This may be jarred severely as was *Mimosa* and the result looked for, the latent period and the temperature being noted, also the period required for regaining the expanded condition.

(*b*) Fully expanded leaves may be explored with a thin rod of glass, or wood, or metal, and the attempt made to locate the area of greatest sensitiveness.

(*c*) Determine whether the strength of the stimulus has any relation to the time and intensity of response. To accomplish this result, stimulate some wholly expanded leaves by striking the sensitive area gently with a glass or metal rod, while corresponding areas of similar leaves are struck severely. Note the latent period in each case.

4. **Experiments with Tendrils.** (*a*) Each student may select some tendril bearing plant growing in the open. He may then, by fastening wire or small wooden rods in suitable places, determine the sensitive area of the tendril, the latent period (the temperature being noted), the mode of action, and the relative age of tendrils responding best to the stimulus.

(*b*) Five grams of gelatine are to be dissolved in 45 c. c. of warm water. A

glass or wooden rod approximately 15 cm. long is to be coated for half its length with hot gelatine. When the gelatine on the rods has solidified, the rods are to be kept free from dust by enclosing them in stoppered test tubes or bottles. While the coats of gelatine are still soft, the tendrils of the passion vine (*Passiflora*) are to be experimented with. First the concave side of the hook of an uncoiled tendril is to be stroked gently three or four times with the gelatine coated rod, and the tendril watched for three or four minutes for a response. If no response is given, the test may be repeated with a naked rod. Determine in this way the irritability of tendrils toward solid and non-solid bodies. What is the biological significance of such a relation? Determine also the latent period, noting the temperature, the progressive coiling or straightening of the tendrils after a very brief stimulation, the extent of sensitive surface of the tendril. Part of the observation for the last topic named may be made on a plant whose tendrils have already grasped supports.

(c) Examine a woodbine (*Ampelopsis*) and find two kinds of climbing organs. Discover the conditions for the formation of each kind.

(d') Find some plant, such as a cucurbit, whose tendrils make spiral coils. Give a description of these coils, noting their various features.

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## Laboratory Outlines for the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

XLIV. (b) *Ricciocarpus natans* (L.) Corda. (Continued.)

7. Make a diagram in the notes as shown in Fig. 7, which represents the general life cycle for all plants above the thallophytes.

XLV. *Marchantia polymorpha* L.

Class, Hepaticæ. Order, Marchantiales. Family, Marchantiaceæ.

This thalloid liverwort is common on moist rocks and earth, especially on cliffs and around springs. *Marchantia* as well as *Conocephalus* and *Lunularia* can be kept without any trouble in a greenhouse or window garden, provided they are supplied with sufficient moisture and shaded from intense light by a curtain.

*Gametophyte.*

1. Take a thallus (frond) and notice its dorsiventral position on the ground. Make a naked eye sketch, showing the dichotomous branching, the central groove, and the emarginate growing points. Describe. How is it fastened to the ground? How does the thallus continue its development? How is vegetative propagation accomplished?

2. Under dissecting microscope study the upper surface. Notice that it is mapped off into diamond-shaped areas (areolæ), each with a small opening in the center (air passage). Draw a patch of the surface.

3. Study the upper surface under low power without a cover-glass, by simply laying the thallus on the slide. Draw several areolæ carefully. The areolæ represent compartments or cavities in the upper surface of the thallus. The thallus should be kept moist on the under surface as it withers very rapidly.

4. Notice the numerous rhizoids on the under surface. Where are they the most numerous? Mount some in water and draw three types under high power—one with smooth wall, one with scattered peg-like projections in the interior, and one with somewhat spirally-arranged projections. Of how many cells does each rhizoid consist? Of what use are the rhizoids?

5. With a scalpel or knife cut off some of the minute ventral scales arranged in two parallel rows on the under side and forming the central ridge. Mount and draw.

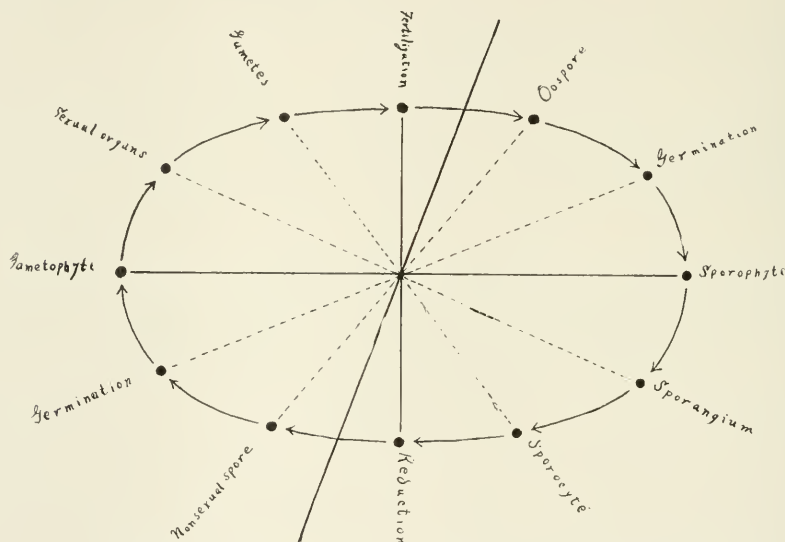


FIG. 7.—Diagram showing principal stages in the life cycle of the higher plants.

6. Some of the thalli will show brood-bud cups. Under dissecting microscope or low power draw one of the cups containing brood-buds (gemmæ). This is a special method of vegetative propagation.

7. Mount some of the brood-buds. Notice the two opposite growing points and the place where the brood-bud was attached to its stalk.

8. Cut cross sections of a thallus through a cup, with a razor, and under high power study the development of the brood-buds. Describe.

9. Cut cross sections of the thallus and examine under high power. Where is the main part of the chlorophyll? Draw part of a section, showing the walls of the cavities below the areolæ, the peculiar chimney-like air passage, the short filaments containing the chloroplasts, and the cellular tissue below the cavities. These details can be worked out better from prepared slides, which should be studied if available and the free hand sections used merely for comparison.

10. Reproduce branches. Draw a plant with an archegoniophore and one



with an antheridiophore. Describe both. Is *Marchantia* hermaphrodite or unisexual?

11. Under high power study prepared slides of cross sections (cut at right angles to the surface of the disc) of the antheridiophore. Draw an antheridium, showing the wall, stalk, and the numerous minute cubical cells in the interior, each of which will produce a spermatozoid. About how many cells in each antheridium? How many antheridia in each disc? Do they all develop at the same time? Notice the openings to the pockets in which the antheridia are situated. About how many spermatozoids are there produced in one antheridiophore?

12. In case no prepared slides are available, cut free-hand sections, mount in water, and study and draw the antheridia under low and high power.

13. From prepared slides study sections of the archegoniophore. Draw an archegonium, showing the lid cells, the neck, the neck canal, the venter, the

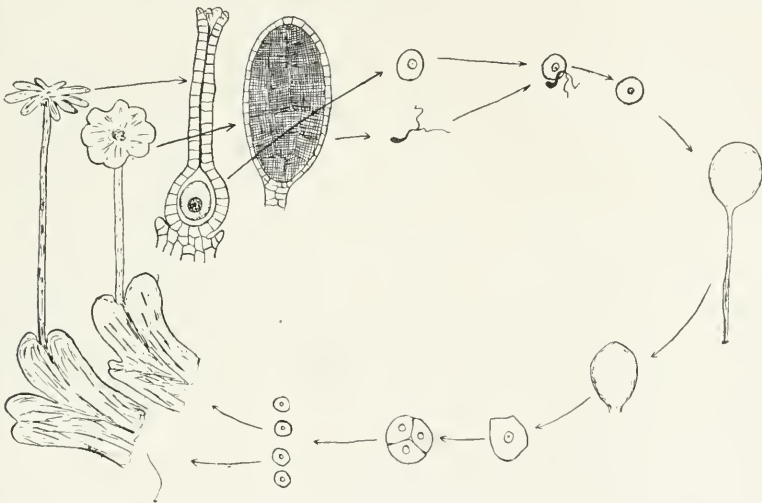


FIG. 8.—Diagram of Life Cycle of *Marchantia*.

oosphere and the inception of the perigynium (incipient perigynium). Draw the venter of an archegonium showing the ventral canal cell and the incipient oosphere.

14. In case no prepared slides are at hand cut sections of the appendaged disc of the archegoniophore, mount in water, and study the archegonia under low and high power.

15. If male *Marchantia* plants with properly developed antheridiophores are protected for several days in such a manner that no water will come onto the discs containing the antheridia, active spermatozoids may be obtained in the following manner. Place a drop of water on the upper surface of the disc and after a short time take it up with a medicine dropper and mount. Under high power numerous motile spermatozoids can be seen, each with two flagella. Study their motion for some time, then stain with a small drop of iodine solution and draw.

*Sporophyte.*

16. Carefully pick out a young light-colored sporophyte inclosed in the perigynium, an older one which appears green, and a nearly mature, yellow-colored one from the under side of the archegoniophore, mount in water and draw under low power, showing the capsule (sporangium), stalk, and foot. Describe.

17. Under high power draw sporocytes hanging together in chains and spore tetrads from crushed capsules, also some mature spores. Draw one of the elaters. What is their function?

18. Make a diagram in the notes showing the life cycle of *Marchantia*. See Fig. 8.

19. Ecological note. Describe how the air passages and the character of the nonsexual spores show that *Marchantia* is adapted to an aerial habitat.

XLVI. *Appendix to Marchantia.*

(a) *Conocephalus conicus* (L.) Dum. Family, Marchantiaceæ.

1. Study the thallus of *Conocephalus* and compare in general with *Marchantia*. Draw.

2. Under dissecting microscope, draw part of the surface showing the areolæ with air passages. How do they compare in size with those of *Marchantia*?

3. Under low power, without cover-glass, draw an areola showing the crater-like air passage. Does *Conocephalus* have any brood-bud cups?

(b) *Lunularia cruciata* (L.) Dum. Family, Marchantiaceæ.

1. Study the thallus of *Lunularia* and compare with *Marchantia* and *Conocephalus*. Notice especially the numerous semilunar brood-bud cups.

2. Draw a plant under the dissecting microscope, showing several cups.

3. Under low power draw several areolæ. How many methods of vegetative propagation has *Lunularia*? Is there much need for sexual and nonsexual spore reproduction?

XLVII. *Porella platiphylla* L. (Bellincinia).

Class, Hepaticæ. Order, Jungermanniales. Family, Jungermanniaceæ.

This rather large, scaly liverwort is very abundant on the bark of trees. It may be kept for a long time in good condition in a paper box.

*Gametophyte.*

1. Moisten a branch of the frond in water and sketch from the upper or dorsal side under the dissecting microscope, showing the arrangement of the lateral scales.

2. Pick off some scales, being careful so as not to tear off the small, lower, ligulate lobe which may be seen under the large upper lobe of the scale. Draw under low power, showing both lobes of the scale. How many cells in thickness is the scale? Is there any midrib? Why is this scale not homologous with the leaf of a fern or one of the higher plants? The scales are partly analogous to leaves.

3. Draw a few cells under high power. Of what advantage are the thick walls?

4. Examine the lower or ventral side of a branch under dissecting microscope and note the semicircular ventral scales. Look for rhizoids. Mount one of the ventral scales and draw under low power.

*Sporophyte.*

5. Examine a frond containing little yellowish, club-shaped bodies. These are the sporophytes. Carefully pick out one which has the capsule unbroken and one which shows the wall of the capsule split into four valves. Mount in water and draw both under low power, showing the capsule, the foot and the stalk.

6. Draw a spore and an elater under high power.

7. Compare the thallus of *Porella* with that of *Marchantia*, noting especially the different ways in which the two thalli have been specialized for the work of photosynthesis.

XLVIII. *Sphagnum cymbifolium* (Ehrb.) Hedw.

Class, Sphagna. Order, Sphagnales. Family, Sphagnaceæ.

The peat or bog mosses grow in and near water in swamps, bogs, and other wet places. The species named above is unisexual, the male plant being more slender than the female.

*Gametophyte.*

1. Take a small mass of dry sphagnum, soak it in water and notice the enormous quantity it will absorb.

2. Make sketches of a male and a female frond. Notice that the frond keeps growing at the top and dying below.

3. Sketch a branch under low power, showing the arrangement of the scales. Draw a single scale. Is there any costa (midrib)?

4. Under high power draw a patch of cells from a scale, some with chlorophyll and some showing the peculiar spiral and ring-shaped thickenings on the inner surface of the wall.

5. Mount a piece of the main stem in water and examine under low power. Draw, showing a central brown cylinder and a cortical layer of clear, large cells with spiral thickenings.

6. Cut off some of the clavate branches at the tip of the male plant, mount, and sketch under low power. Pull off the scales carefully, mount, and examine the antheridia. Draw an antheridium under high power.

7. From a female plant carefully cut out an enlarged archegonium containing a young sporophyte. Mount and draw under low power, showing the neck at the summit. Around the base some small archegonia may usually be seen. Draw one of these, showing the stalk, venter, neck, and lid cells.

*Sporophyte.*

8. Pick out a young sporophyte showing the spherical capsule, the very short stalk and the expanded bulbous foot.

9. Cut off one of the slender pseudopodia containing a nearly mature sporo-

phyte. Sketch under low power showing the sporophyte with capsule and sperculum, and the expansion at the top of the pseudopodia into which the foot fits.

10. Draw some of the nonsexual spores under high power.

11. From prepared slides make a drawing of a longitudinal section of the sporophyte, showing all the details of the structure.

12. Study and draw an apical cell from a branch of the gametophyte, from a prepared slide.

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## Bacteriology for High Schools.

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### V.

#### MICROSCOPICAL EXAMINATION OF BACTERIA—Continued.

The bacteria are very widely distributed in nature and are found in practically all parts of the earth, except in the air in mid-ocean and in that on the tops of the highest mountains. By means of the potato culture already described it would be possible to determine facts in regard to their variety and distribution. It will, however, be found more satisfactory to use for this purpose, not potato cultures, but gelatine cultures.

*Preparation of Gelatine Culture Medium.* This method is essentially a beef broth, or bouillon, to which enough gelatine has been added to cause it to solidify.

A teaspoonful of extract of beef such as Leibig's or Armour's is added to a pint of water which has been placed in the inner vessel of a double walled cooker. In case the extract of beef is not readily obtainable one-half pound of lean beef, as round or shoulder steak, is purchased and cut up fine (minced) and put to soak in a pint of water over night in a cool place. In the morning the juice is squeezed out by straining through a cloth and enough water added, if necessary, to make a pint. This meat juice is now placed in the cooker as above. It ought, perhaps, to be stated that the latter method is always the best, but that the other is much easier to prepare, is sufficiently good and is, therefore, given first.

To the meat juice is now added two ounces of gelatine. One of the gelatines for sale at all of the grocery stores will do, although it is best to have a sheet gelatine. This amount is intended to make a 10 to 12 per cent. gelatine. The water in the outer dish is now brought to a boil and the heat continued until the gelatine is all in solution. The gelatine is very acid and must be neutralized. It is best done at this point. It is most conveniently done by adding ordinary baking soda or saleratus. This should be added in very small amounts and after such addition a strip of red litmus paper should be dipped into the medium. At first the paper will not be changed in color, but as the additions continue a point will be reached where the red paper will be turned a faint blue. If it is tested at this point with a piece of blue paper the color will not be



changed at all or will be turned only to a slightly deeper blue. The process of neutralization should be carefully performed. Care must be taken to stir thoroughly after each addition of soda. Only a small strip of litmus paper need be used, since the portion wetted each time can be easily torn off and the remainder used again.

After neutralization the gelatine is boiled for a few moments and then cooled down to  $60^{\circ}\text{C}$ . ( $140^{\circ}\text{F}$ .) and an egg is added. If beef was used it will not be necessary to add the egg, but if the extract was used it is best at this point to beat an egg with a little water, add it to the gelatine and, after thoroughly mixing, heat as hot as possible in the double dish and continue until the egg is thoroughly coagulated, i. e., cooked into a solid mass. It is best not to stir during the cooking.

When the egg is thoroughly coagulated the gelatine is to be filtered through a little absorbent cotton (ordinary cotton will not do) in the neck of a funnel as shown in Fig. 18. The filtration will be quite rapid if the egg was thoroughly cooked, otherwise it will be slow, sometimes distressingly so. In the latter case it must be kept in a warm place, as in a steam-bath, until the filtration is complete. When the gelatine is filtered it should be quite transparent. The clearing and filtration require patience. They may be omitted, but in that case the gelatine will be more or less opaque and unsatisfactory.

One of the most important things to remember in the preparation of gelatine is that the continued application of heat injures its solidifying powers, and that if the heat is applied too long it will not harden on cooling. The greatest care, then, must be exercised to heat hot while necessary and then stop as soon as the purpose of the heating has been accomplished.

After the gelatine has been filtered it should be distributed into test-tubes or bottles, if the laboratory is supplied with test-tubes and Petri dishes (see Fig. 6). In other cases the gelatine is best put in flat bottles, such as those shown in Fig. 5. The amount to be put in each tube or bottle is such that it will form a layer about an eighth of an inch deep in the Petri dish or on the side of the bottle. The glassware cannot, however, be used for this purpose until it has first been sterilized. And before sterilization it must be plugged with cotton. For this purpose ordinary cotton wool can be used, but absorbent cotton is better. The cotton plugs should be rolled so that they will be compact, and so made that when in place they will leave no creases for the entrance of dust. They should not be put in too tight, only sufficiently so to prevent their being readily pulled out. The general rule is tight enough to support the weight of the vessel and its contents. Glassware is sterilized in the hot-air sterilizer at  $150^{\circ}\text{C}$ . for one hour, or where the thermometer is not available the heat is applied until the cotton begins to turn brown, i. e., to scorch. At this point

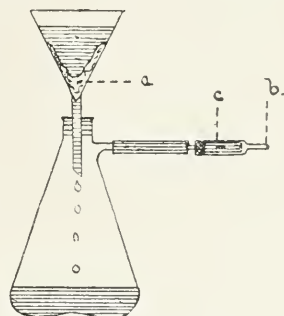


FIG. 18.—Apparatus for filtering media through absorbent cotton: *a*, layer of cotton; *b*, tubes for making connection with air pump; *c*, Bunsen valve to prevent entrance of water into flasks.

sterilization may be safely stopped. The sterilization of the glassware should be done either before the preparation of the gelatine is begun or while it is being made, so that the vessels will be ready to receive the gelatine as soon as it is filtered.

When the gelatine has been distributed into test-tubes or bottles it must be given a final sterilization. This is done in the steam sterilizer and never in the hot-air sterilizer, where it would only be evaporated and charred. The steam sterilizer may be either an Arnold or the simpler form shown in Fig. 2. In such a sterilizer the gelatine is placed and the water quickly brought to a boil; the material is allowed to stay in the steam for twenty minutes and then removed and cooled. It is allowed to stay at the temperature of the room for twenty-four hours, when it is again heated as in the first instance and again allowed to stand, and heated for the last time on the third day. This method is known as the discontinuous method of sterilization. The theory is that on the first day all of the bacteria are killed which are not in a spore form. The spores it would be impossible to kill with steam unless the exposure extended over several hours, which would injure the gelatine. While in the media, the spores which were not killed germinate and the next day the young forms are readily killed. If any should not be destroyed they are killed the third day. The gelatine should be kept in a cool, dark place until it is used. If a cloudiness or specks appear it means that it is not sterile and must be discarded.

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According to Brinckerhoff and Tyzzer and Professor G. Sims Woodhead, the following wet method for blood films gives excellent results:

1. Hold the wet film, wet side down, in the mouth of a wide bottle, half filled with ordinary 40 per cent. solution of formic aldehyde, for about five seconds.
2. Drop, still wet, film side downwards, into absolute alcohol. Leave fifteen minutes, or, if more convenient, as long as forty-eight hours.
3. Blot off excess of alcohol, and move cover-glass to a dry part of the blotting-paper.
4. Immediately, before any drying occurs, drop on a few drops of eosin-methylene blue stain (Jenner's stain); cover with a watch-glass; stain for two minutes, no longer.
5. Allow excess of stain to run off the cover-glass, and rinse at once in a bowl of distilled water.
6. Blot off excess of water.
7. Dehydrate very rapidly in absolute alcohol, merely dipping in and withdrawing as quickly as possible.
8. Wash off alcohol in first xylol rapidly; wash in second xylol; drop on fresh xylol.
9. Mount in xylol balsam.

Scott insists upon the importance of using only pure distilled water to wash off excess of stain and cover-slips which are quite free from acid. The blood film must not be allowed to dry at any stage.—*Journal of State Medicine.*

## LABORATORY PHOTOGRAPHY.

L. B. ELLIOTT.

Devoted to Methods and Apparatus for Converting an Object into an Illustration.

### The Lantern in Class Room of Pharmacognosy.

The primary object of Pharmacognosy, as more especially adapted to the needs of students of pharmacy, is to enable him to recognize drugs, to determine their quality, to detect their adulteration and to distinguish the characteristic elements of those to which they are closely allied. The study of the development of different organs of plants and of histological changes of their growth and the botanical relations of orders, genera and species, while helpful and important in many ways, is not so directly applicable to his needs as the study of histological elements, by the aid of the microscope.

This microscopical study has been applied especially to powdered drugs and in this work considerable progress has been made within the past few years. It is now quite possible to identify most of the official drugs of the *materia medica*, even in the powdered form, when the gross characteristics have disappeared in the progress of pulverization. This has been accomplished by the faithful workers who have devoted much time to the study of what may be termed the inner morphology and the investigation of the air dry material as it exists in the somewhat changed vegetable cell—changed in the process of dessication.

As evidence of this work there have been most excellent papers and monographs upon powdered drugs published, and during the past few years, also, there have been issued text-books and volumes upon the subject, such as: "Powdered Vegetable Drugs," by Albert Schneider, M. D., Ph. D.; "A Course in Botany and Pharmacognosy," by Henry Kraemer, Ph. D., Ph. B.; "Microscopical Examinations of Foods and Drugs," by Henry George Greenish. Last year there was completed the "Anatomischer Atlas, Der Pharakognosie und Nahrungsmittelkunde von Dr. A. Tschirch und Dr. O. Oesterle."

Formerly it was considered sufficient for identification of vegetable drugs to describe gross characteristics only, such as color, odor, taste, and such other characters as might be brought out by the simple microscope, magnifying, say ten to fifteen diameters, but this method has become inadequate because of the new factor in drug supplies—the drug miller. He supplies these in powdered form, and the process of pulverization the druggist is glad enough to transfer to those who have laboratories especially provided for that purpose. But it is easy to see that adulteration is made easier and its detection more difficult, hence it is necessary to teach every student in pharmacy methods of microscopical analysis of drug powders.

In order to detect adulteration in a vegetable powder it is necessary to become thoroughly acquainted with the structure of the drug of which the powder is made and to have a knowledge of the different elements contained in the

drug. If the powder be the product of a bark or a wood, a leaf, a root, or a seed, the elements common to each one of these should have been studied and well understood. Wood fibres, if found to any extent in a powder of a bark, would naturally be regarded with suspicion. Bark elements found in powdered leaves would likewise cause suspicion to rest upon the preparation under consideration. But if the adulteration consists of similar elements of the same part of the plant, the problem becomes more difficult. Very close examination of the structure of the elements of the drug under consideration is then absolutely necessary. It is not infrequently the case that substances of a similar kind but of inferior and cheaper varieties are used, as in the case of Ceylon cinnamon, which may be adulterated with the inferior variety known as the Saigon cinnamon. Under the microscope we find in the Ceylon cinnamon an absence of cork cells: the stone cells are more elongated and unevenly thickened than in the other variety. These and other less important characteristics enable one to determine the character of the powder, and to detect admixture. Schneider calls attention to the admixture of *Apocynum cannabinum* with *A. androsæmifolium*: *Surinum quassia* with *Jamaica quassia*. These resemble each other as to gross appearances, but the histological characters are quite different. *A. androsæmifolium* is distinguished from the other species by the presence of stone cells. The *Surinum quassia* differs from the other in the form of the medullary rays and the presence of stone cells in the bark. Powdered senega has been adulterated with soap bark. This latter may be detected by the presence of readily distinguished liber fibers only made manifest by the use of the microscope.

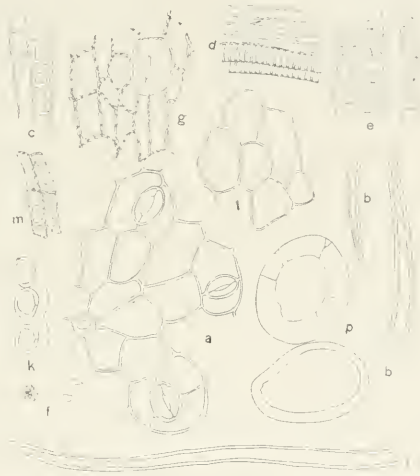
It is clear that the student in pharmacy should be well drilled in the histological elements of vegetable powders and be made familiar with the use of proper microscopical reagents. The laboratory work should be supplemented by lecture room demonstration. Here we find the lantern to be of great importance and utility. A lantern for this purpose should be provided with an easily adjusted microscopical projection as well as a projection for photographic slides. In the preparation of slides for demonstrating the elements in powdered drugs we find considerable difficulty. Powders cannot be prepared by staining and clearing, as thin sections may be, for projection, and so do not give sufficient contrast to bring out details of structure sufficiently. The lantern may show certain characteristics that are helpful, but these are not entirely satisfactory to instructor or student because, in the case of powders, the detailed structure cannot be made out at the distance the student must sit from the screen. The object loses its sharp outline and detail in its projection from the objective to the screen. To overcome this drawings can be made of structures under the camera lucida, and from these lantern slides may be made in the usual way. When these are made and used in the lecture room in connection with the laboratory work with the compound microscope the student cannot fail to see the connection and the instructor is decidedly helped in his work.

As an example of such a lantern slide we have prepared and present here one which illustrates a mixture of powdered senna leaves with powdered savine (*Juniperus sabina*). These leafy drugs are totally different in their gross characteristics, but strange to say, in the powdered state have some characteristics



in common. We have known the powder of the one to be mistaken for the other. A lantern slide prepared of the mixture of the two powdered leaves serves only, however, to show how to distinguish the histological elements in a mixed powder, although these elements may be mainly of leaf fragments, and serves also to illustrate the principles of identification by microscopical study of histological elements.

By referring to the figure it will be seen that we have from Alexandria senna : (a) epidermis; (g) epidermis from savine. These epidermal cells when compared, as to the shape and size of the stomata and the shape and character of the epidermal cell walls, furnish a means of distinguishing the two leaves without



MIXED DRUG POWDERS.

- (a) Epidermis showing stomata from Alexandria senna.
  - (b) Trichomes from Alexandria senna.
  - (p) Stone cells from savine.
  - (j) Bast fiber from savine.
  - (l) Parenchyma cells from savine.
  - (e) Parenchyma cells with calcium oxalate crystals from Alexandria senna.
  - (d) Annular ducts and sclerenchyma fibers from Alexandria senna.
  - (g) Epidermis with stoma from savine.
  - (c) Crystal fiber and sclerenchyma from Alexandria senna.
  - (m) Sclerenchymatous cells with bordered pits from savine.
  - (f) Crystals of calcium oxalate from Alexandria senna.
  - (k) Epidermis—lateral view—from savine.
- Magnified, 173 diameters.

effort. If now, we look for other histological elements, first of senna, we see the hairs at (b), the crystal bearing fiber at (c), the parenchyma cells bearing calcium oxalate crystals at (e), and isolated crystals of calcium oxalate at (f). On the other hand, we have from savine sclerenchymatous cells with bordered pits at (m), long, slender bast fibers at (j), and stone cells at (p), which furnish ready means for illustration by which students may easily comprehend the subject and under-

stand the method of study. Powdered digitalis has been more than once mistaken for powdered senna. Mixed powders of digitalis and senna can be very readily distinguished by the microscope, and a lantern slide of this mixture would show much more marked difference of structural elements. In the powder would appear the simple non-secreting hairs consisting of two to five superimposed cells, and hairs with two celled glandular head, very characteristic of digitalis powder.

School of Pharmacy, University of Kansas.

L. E. SAYRE.

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Dr. Kasperek has described a very ingenious funnel devised for the purpose of filtering gelatine or agar that needs to be kept warm during filtering. The piece of apparatus consists of an ordinary glass funnel, in which are placed several layers of asbestos paper, fastened together with water glass, the asbestos paper conforming to the shape of the filter. Between the layers of the asbestos there are wound about 3 meters of a 3 millimeter nickel wire, so wound around that the different coils are thoroughly isolated from each other. The two ends of the wire are connected with binding screws. To one of the binding screws is attached an electric wire from an electric current, and to the other a wire that is arranged to pass through a series of ordinary incandescent lights. By connecting the wires with the electric current and turning on a single light, the filter is warmed to a temperature of  $42^{\circ}$ . By connecting with two lights of similar power the filter is warmed to  $60^{\circ}$ , and by the addition of a third light of 16 candle-power a temperature of  $70^{\circ}$  can be obtained. This filter is used in the ordinary way, and is extremely convenient for filtering material that needs to be kept warm.—*Journal of State Medicine*.

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Quite recently Dr. J. Ruhemann presented to the Berlin Medical Society a new method for the *quantitative* estimation of uric acid in the urine, which is based upon the principle of the union of uric acid with iodine and is carried out in the following manner: The author had constructed a burette, 25.5 cm. in length, with the following divisions: The lowest mark *S* shows the height to which the indicator, sulphid of carbon, should reach. Then follows to the mark *I* a space of 2 c. c. content in which iodine solution is filled. The latter solution is composed of iodine, 1.5; potassium iodide, 1.5; alcohol, 15.0; and water, 185.0. Above the mark *I*, at 2.6, begins an empirical scale which, at distances of 0.2 c. c., gives the uric acid value pro mille. It runs from 2.45 to 1.175 gm. pro mille. After the sulphid of carbon and iodine solution have been placed in the burette, observing the precautions laid down by Ruhemann, the urine is slowly added and the mixture vigorously shaken after each addition. The urine is added until the primary brown color gives place to a white color, at which moment the percentage of uric acid is read off by the aid of the figures at the top of the column of fluid. The procedure requires, on an average, about 30 to 45 minutes. Ruhemann claims for his method rapidity and an accuracy equal to that obtained by the weight analysis method.—*The Post Graduate*.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Miyake, K. On the development of the sexual organs and fertilization in *Picea excelsa*. *Annals of Botany*, 17: 351-372, pls. 16-17, 1903.

The pollen grain at the time of shedding—about the second week in May—contains two disorganized prothallial cells, a stalk cell, body cell, and tube cell. The tube begins to form a few days after pollination and the body cell at once passes into it and divides, giving rise to the two male nuclei. At this division the beginning of a cell plate appears at the equator of the spindle, but it soon disappears and no wall is formed; consequently the two male nuclei lie free in a common mass of cytoplasm and there is no formation of two cells as described by Strasburger, Belajeff, Dixon, and Coulter. The pollen tube does not branch.

The development of the archegonium is very much as in *Pinus*. In the neck of the archegonium there are 4 to 8 rows of cells with 2 to 4 cells in a row. There are usually four archegonia in each ovule, but the number varies from two to seven. During the growth of the egg no passage of nuclear material from the jacket cells into the egg could be detected. The ventral canal cell is formed about a week before fertilization, which, in the neighborhood of Ithaca, occurs about the middle of June. No walls are formed in the proembryo until it has reached the eight-celled stage. Strasburger describes walls at the four-celled stage and other writers have described walls at the four-celled stage in *Pinus*.

The antheridial cell of Strasburger (third prothallial cell of Belajeff) is called the central cell by Dr. Miyake, who regards it as the equivalent of the central cell of Pteridophytes. The "body cell" of Strasburger is called the generative cell. Strasburger refers to the two male cells as "generative cells." The terminology is confusing, and we are not sure that the present writer has been entirely consistent.

C. J. C.

Zacharias, E. Ueber die "achromatischen" Bestandtheile des Zellkerns. *Ber. d. deutsch. bot. Gesell.* 20: 298-320, pl. 16, 1902.

This paper deals with the contents of the nucleus, exclusive of the nucleolus and nuclein-containing structures.

Pollen mother cells of *Larix*, *Iris*, *Hemerocallis* and other forms, were investigated. Material was examined in the living condition and also after treatment with various reagents, but sections do not seem to have been used. In dealing with nuclei in division after the nuclear membrane has broken down, the special term, nuclear cavity (*Kernraum*), is used, because the sphere of influence of the nucleus may not be the same as when the nuclear membrane is intact. The writer does not agree with Nêmec's statement that the spindle fibers consist of plastin, but believes that plastin may be present in some cases while in others it may be lacking.

In the living cell during nuclear division the nuclear cavity, with the excep-

tion of the chromosomes, appears as if filled with a homogeneous fluid in which movable thread-like structures may appear between the separating groups of chromosomes. Prof. Zacharias believes that his own investigations, as well as those of morphologists, show that definite spindle fibers have not yet been demonstrated in the living cell and that it is possible that the structures seen in fixed material may be artifacts.

C. J. C.

Hegelmaier, F. Zur Kenntniss der Polyembryonie von *Euphorbia dulcis* Jacq. (*purpurata* Thuill.). Ber. d. deutsch. bot. Gesell. 21: 6-19, pl. 2, 1903.

Prof. Hegelmaier's preliminary announcement of habitual polyembryony in *Euphorbia dulcis* has already been noted in the JOURNAL (p. 2058, Nov. 1902). In the present paper, which is accompanied by figures, the previous observations are confirmed. The more extended investigation shows that about three-fourths of the ovules contain more than one embryo. A considerable percentage of the pollen is sterile and fertilization was not observed, although it probably occurs in many cases. Pollination is not necessary for the production of adventitious embryos, at least not for those coming from the nucellus. Whether an embryo would develop from an egg without fertilization, was not determined. The writer withdraws his earlier statement that polyembryony, as found in *Euphorbia*, might lead to apogamy and even to parthenogenesis.

C. J. C.

Tischler, G. Ueber eine merkwürdige Wachsthumerscheinung in den Samenanlagen von *Cytisus Adami* Poir. Ber. d. deutsch. bot. Gesell. 21: 82-89, pl. 5, 1903.

Irregular mitoses in the pollen mother cell and the consequent formation of imperfect pollen has already been noted in several sterile hybrids. In *Cytisus adami*, a hybrid between *Cytisus laburnum* and *C. purpureus*, the development of the pollen is regular, but abnormalities which result in sterility are found in the ovule. After the integuments are quite well developed, a region at the base of the nucellus, rich in protoplasm, begins to grow with great rapidity, so that the nucellus is soon forced out through the micropyle. Often no megaspore mother-cell can be detected, sometimes a larger cell with shrunken protoplasm and a few nuclei indicates that a megaspore mother-cell had begun to germinate, and occasionally, when the nucellar growth is not particularly extensive, a normal embryo-sac may appear.

C. J. C.

Swingle, Deane B. Formation of the spores in the sporangia of *Rhizopus nigricans* and *Phycomyces nitens*. U. S. Dept. of Agric., Bureau of Plant Industry. Bulletin No. 37, pp. 1-40, pls. 1-6, 1903.

Pure cultures were obtained and the material was then fixed in various reagents. Particularly fine preparations were obtained from material fixed for one hour in Flemming's fluid followed by twelve to twenty-four hours in Merkel's fluid or chrom-acetic acid. Sections were cut  $2\mu$  to  $4\mu$  in thickness and were stained in the safranin, gentian-violet, orange combination.

The paper deals especially with the mechanics of this peculiar cell division found in these sporangia and with the nature and functions of the vacuole. It is of interest to note that the four genera of the Mucorineæ which have been carefully investigated—*Pilobolus* and *Sporodinia* studied by Harper, and *Rhizopus* and *Phycomyces* studied by the present writer—differ considerably in the forma-



tion of their spores. The following is Prof. Swingle's own summary of the process of spore formation in *Rhizopus* and *Phycomyces*:

1. Streaming of the cytoplasm, nuclei and vacuoles up the sporangiophore and out toward the periphery, forming a dense layer next the sporangium wall and a less dense region in the interior, both containing nuclei.

2. Formation of a layer of comparatively large, round vacuoles in the denser plasm parallel to its inner surface.

3. Extension of the vacuoles by flattening, so that they fuse to form a curved cleft in the denser plasm; and, in the case of *Rhizopus*, the cutting upward of a circular surface furrow from the base of the sporangium to meet the cleft formed by these vacuoles, thus cleaving out the columella.

4. Division of the spore-plasm into spores; in *Rhizopus*, by furrows pushing progressively inward from the surface and outward from the columella cleft, both systems branching, curving, and intersecting to form multinucleate bits of protoplasm, surrounded only by plasma-membranes and separated by spaces filled with cell sap only; in *Phycomyces*, by angles forming in certain vacuoles containing a stainable substance and continuing outward into the spore-plasm as furrows, aided by other furrows from the columella cleft and dividing the protoplasm into bits homologous with and similar to those in *Rhizopus*, and separated by furrows partly filled with the contents of the vacuoles that assist in the cleavage.

5. Formation of walls about the spores and columella, and, in the case of *Rhizopus*, the secretion of intersporal slime.

6. Partial disintegration of the nuclei in the columella.

C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE MOODY.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole Moody, 125 Belvedere Street, San Francisco, Cal.

Brues, C. T. Development of Stylopide. Zool. Jahrb. 18: 241-270, 2 pls. 3 figs., 1903. Rev. J. R. Micr. Soc. Pt. 4, p. 494-495, 1903.

The author has worked on three North American species of *Xenos*; these live as internal parasites of wasps, especially *Polistes*.

The behavior of infected wasps toward the parasites is usually friendly. The distribution is erratic, due probably to the difficulty of transfer of parasites from one nest to another. Large numbers of larvæ occur in one host, which dies soon after the emergence of the male *Xenos*. The oögenesis is peculiar. Very small larvæ show strings of primitive ova on each side of the gut; these grow and later break up, giving rise to eggs, each of which consists of a mass of muse-cells with a polar cap of cells divided from the primitive egg. Yolk is formed from the contents of each egg, and when mature, eggs are scattered through the body and in the fat body. Maturation apparently occurs through

the fusion of the second polar body and the pronucleus of the egg. Cleavage forms a blastoderm which covers one pole, making a rudiment of a germ band by multiplication and rearrangement of cells. The first larvæ stage or "*triangulin*" gives rise to the second or legless larva by the degeneration of internal organs, median metameric protuberances taking the place of legs. The sexes differ in external form after the first moult. No affinity with the coleoptera is indicated.

A. M. C. M.

**Bohn, G.** Influence of Radium on Tadpoles. *Compt. Rend.* **136**: 1012-1013, 1903. *Rev. J. R. M. S.* **4**: 483, 1903.

These rays have a distinct but variable effect on the growth of the tadpoles of frogs and toads, in slowing, quickening, destroying or perverting development. An effect produced during the tadpole life may remain latent till metamorphosis, when monstrosity suddenly results.

A. M. C. M.

**Bohn, G.** Influence of Radium Rays on Ova. *Compt. Rend.* **136**: 1085-1086, 1903.

Over forty experiments were made by placing the ova of *Strongylocentrotus lividus* near a tube of radium. Apparently the rays acted on the chromatin of the nucleus, increasing its activity or afterwards destroying it. Spermatozoa (almost naked chromatin) are destroyed, but the protected chromatin of ova is excited and parthenogenesis induced. There seem to be no effects on tissue except during differentiation.

A. M. C. M.

**Ziegler, H. E.** Influence of Alcohol on Development. *Bull. Centrbl.* **23**: 448-455, 5 figs., 1903. (*Rev. J. R. M. S.* **4**: 484, 1903.)

Ova of the sea urchins *Echinus microtuberculatus* and *Strongylocentrotus lividus* were used to test the effect of alcohol during development. The presence of 0.5-1 per cent. causes no serious injury, normal plutei may result, but individual susceptibility is markedly different. The presence of 2 per cent seriously disturbs development and acts as poison. Cleavage is slow, often abnormal, few blastulæ are formed and the blastocœle tends to be too small and mesenchyme cells too numerous. Gastrulation is sluggish and mesenchyme cells disarranged; any skeleton formed is abnormal, plutei have poorly developed arms. With 3 per cent. in the sea water few blastulæ are formed and no gastrulæ. With 4 per cent. no blastulæ appear. The general result is inhibition of cell-division; nuclear division may occur without cell division. Cell movements are also inhibited as shown in the modified gastrulation.

A. M. C. M.

**Marino, F.** Non-existence of "Neutrophili" Granules in Leucocytes of Man and Monkey. *Am. Instit. Pasteur*, **17**: 357-364, 1 pl. 1903. (*Rev. J. R. M. S.* **4**: 486, 1903.)

Erlich's classification of leucocyte granules is not applicable to man and monkeys, according to the author. Erlich's groups are eosinophil (acid staining), basophil (basic staining), neutrophil (neutral staining). Marino finds only the first two present, the so-called neutrophil granules stain with either acid or eosin stains and retain them.

A. M. C. M.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

Rubaschkin, W. Zur Morphologie des Gehirns der Amphibien. Arch. f. mik. Anat. u. Entw. 62: 207-244, Taf. 12, 13, 1903.

The brains of *Rana*, *Bufo*, *Salamandra* and *Triton* were studied by impregnation processes with some modification

of wellknown methods. The blood system of the animals used was injected either *intra vitam* or *post mortem* with a 5 per cent. solution of potassium bichromate, or with Lavdowsky's saturated solution of neutral chromate of potash or rubidium. Ten or fifteen minutes after injection the nervous system was removed, cut into pieces .5 to 1 cm. in length and placed for 12 to 18 hours in the following mixture, made just before using.

5 per cent. bichromate of potash	-	-	-	100
1 per cent. osmic acid	-	-	-	15
40 per cent. formaldehyde (Schering)	-	-	-	5

This mixture darkens in 24 hours after mixing and loses its efficacy as a fixing agent. Clearness of the preparation is also enhanced if acetic acid to a strength of 1 per cent. is added to the mixture. The superficial layers of the tissue will then be found free from precipitates. If, however, the acetic acid is added to the injecting fluid also the tissues throughout will be freed from precipitates. Impregnation in a strong solution (2 per cent.) of silver nitrate for 8 to 12 hours was followed by the usual embedding in celloidin. For clearing purposes turpentine concentrated by evaporation to a syrupy consistency was used so as to avoid the shrinking which follows the use of xylol or toluol.

C. A. K.

Neidert, L. und Lieber, A. Ueber Bau und Entwicklung der weiblichen Geschlechtsorgane de Amphioxus lanceolatus. Zoöl. Jahrb. Abth. f. Anat. u. Ont. 18: 187-227, Taf. 15-19, 1903.

The size of the animal is here no criterion to the stage of sexual development. Small individuals 20 to 40 millimeters in length have been found

with matured sexual cells, while larger specimens were often immature. The best fixing agent was found to be sublimate acetic. It caused little rupture of tissues and less shrinkage than fluids containing picric acid, after which the epithelium was frequently torn so as to render the material worthless. The regions containing the ovaries were cut away with scalpel upon hardened liver, stained, cleared, and studied *in toto* prior to sectioning. Double staining in borax-carmin and Delafield's hæmatoxylin was employed. Nuclei took the carmine stain, while blood, yolk, and membranes were stained by the hæmatoxylin.

C. A. K.

Kotte, E. Beiträge zur Kenntniss der Hautsinnesorgane und des peripheren Nervensystems der Tiefsee-Decapoden. Zoöl. Jahrb. Abth. f. Anat. u. Ont. 17: 619-659, Taf. 23-27, 1903.

The author describes in this paper the method he has employed in demonstrating the sense organs of some fresh water *Crustacea*. Only customary staining

methods were used on the material with which the paper deals. Fresh water

forms such as *Cyclops*, *Branchipus*, *Daphnia*, *Gammarus*, and *Asellus* were placed for 1 to 2 days in a faintly colored solution of methylen blue. Without exception they live in such solutions without disaster. After removal from the solution they were washed in water and examined under cover-glass. In *Daphnia* the antennal sense organs, the ventral nerve chain, and the post abdominal sense organs are clearly demonstrated by this method. Attempts to apply the Golgi method were fruitless.

C. A. K.

**Goldschmidt, R.** Histologische Untersuchungen an Nematoden, I, Die Sinnesorgane von *Ascaris lumbricoides* L. und *A. megalocephala*. Cloqu. Zööl. Jahrb. Abth. f. Anat. u. Ontog. 18: 1-57, Taf. 1-5, 1903.

The parasites were taken directly from the intestine of the host and placed in the fixing fluids. Histological preservation was poor (because of the thick cuticula) if the entire animal was placed in the fluid. It was therefore desirable to cut the worms into small pieces. This is contrary to the experience of Apathy, who recommends fixing the *entire* worm in hot sublimate. The author finds, however, that the nemofibrillæ are best demonstrated in material which is otherwise in a state of poor histological preservation. Best results were obtained with sublimate-acetic (sublimate  $\frac{1}{2}$  conc. + 2 per cent. acetic), and the following were found useful: concentrated sublimate, Perenyi's fluid, and acetic-alcohol (70 per cent. alc. 4 parts + 1 part 43 per cent. acetic acid). Picro-sulphuric, picro-acetic, chrom-acetic, and Hermann's fluid gave poor results. Chloroform-paraffin method of infiltration was employed and sections 2 to 6  $\mu$  in thickness were used. The sense organs of the anterior end were best studied in frontal sections. *In toto* staining with Heidenhain's chrome-hæmatoxylin gave a very intensive and at the same time transparent stain. Pieces of tissue were stained for 12 hours in  $\frac{1}{2}$  per cent. aqueous hæmatoxylin followed by 1 per cent. chromate of potash for an equal length of time. Delafield's hæmatoxylin followed by eosin gave excellent detail, as did also Van Gieson's method. Apathy's gilding method and methylen blue *intra vitam* were tried without success.

C. A. K.

**Weber, A.** L'origine des glandes annexes de l'intestin moyen chez les vertébrés. Arch. d'Anat. Micr. 5: 485-727, pls. 17-27, 1903.

Embryos studied were those of *Miniop-terus schreibersii* fixed for several days in bichromate-acetic, of *Anguis fragilis* fixed in sublimate acetic or in Zenker's fluid, and of the duck fixed in various sublimate mixtures—Carnoy's, Zenker's, and sublimate-acetic. Duck embryos fixed in these sublimate mixtures are quickly hardened and exhibit a minimum of folding or dislocation of parts. For advanced stages, after formation of the umbilical region, fixing fluids based on bichromate of potash, such as bichromate-acetic or Zenker's fluid, preserve the organs in proper relations. To obviate the difficulties arising from sublimate in Zenker's fluid the author places embryos of 5 to 6 days incubation in this fixing agent for 12 hours only, then without washing placed them directly in bichromate-acetic for several days, changing the fluid several times. In this way the sojourn of these large embryos in iodine-alcohol is materially reduced. Embryos intended for use in graphic or plastic reconstruction were overstained in borax carmine or Mayer's alcoholic carmine.

Outlines of embryonic organs such as the notochord or neural tube were



used for purposes of plastic reconstruction by Born's method in place of planes of definition. To remedy the deformation caused in reconstruction by irregularities in the thickness of the superposed plates, the author arranged them in divisions of equal numbers and pressed them in a vise until they were reduced to the thickness demanded by the magnification.

A new form of graphic reconstruction designed to show the *relative thickness* of various parts of the intestinal epithelium is described by the author. Upon a surface drawing of the desired object made at right angles to the plane of the sections there are projected all joints in which the intestinal epithelium has the same thickness. These thicknesses are measured upon drawings of the sections magnified to the same scale as the surface drawing. With a magnification of 200 diameters, variations of  $5\mu$  in thickness were followed in the sections and plotted on the surface drawing. By joining the points thus established the curves which limit the fields of intestinal epithelium, differing by  $5\mu$  in thickness, can be mapped out on the drawing and denoted by depth of tinting. The direction of the axes of spindles of dividing cells was also indicated by short lines when parallel to surface, or by dots when perpendicular to it. Regions of thickening and areas of cell proliferation are thus graphically presented with great effectiveness.

C. A. K.

**Dogiel, A. S.** Nervenendigungen in der Pleura des Menschen und der Säugetiere. Arch. f. mik. Anat. u. Entwickl. **62**: 244-251, Taf. 14, 1903.

In addition to human tissues, those of the dog and cat were studied. A  $\frac{1}{4}$  to  $\frac{1}{6}$  per cent. solution of methylen blue was injected into the blood vessels

or a  $\frac{1}{6}$  to  $\frac{1}{8}$  per cent. introduced into the pleural cavity. After 20 to 25 minutes the sternum and the cartilaginous portions of the 2d and 4th ribs or even the whole ribs to their attachment to the vertebræ were removed together with the intercostal muscles and the pleura. The preparations were then placed in glass vessels in a thermostat at  $36.5^{\circ}$ - $37^{\circ}$  C. for 25 to 40 minutes or even 1 to  $1\frac{1}{2}$  hours. Fixation of the methylen blue stain was accomplished in a considerable volume of 5 to 8 per cent. ammonium molybdate. After 18 to 24 hours the pleura was dissected away together with a part of the internal intercostal muscles, washed for 2 to 3 hours in distilled water and still further freed from the muscles so that the preparations were thin enough for investigation. Pieces of the pleura several centimeters in size were pinned out on cardboard, dehydrated, freed from the cardboard, cleared in xylol and mounted in xylol-damar. Nerve endings in pleura, at the transition of muscle and tendon and on the muscles themselves, can then be studied.

C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoological Laboratory, University of Michigan, Ann Arbor, Mich.

Clowes, G. H. A. The Relationship between the Freezing Point Depression and Specific Gravity of Urine, under Varying Conditions of Metabolism, and its Clinical Value in the Estimation of Sugar and Albumin. Amer. Jour. Physiol. 9: 319-343, 1903.

In this experimental study of the urine of normal and pathological subjects, carried on by the methods of physical chemistry, the author has deduced some valuable constants for practical work

in urine analysis. He finds in agreement with earlier workers that in normal urine the depression of the freezing point is directly proportional to the specific gravity, and may be determined from the latter by multiplying the figures after the decimal point by 75. (As an example, if the specific gravity is 1.022,  $0.022 \times 75 = 1.65$ , which is found by experiment to be the depression of the freezing point of such a solution in degrees C.) In pathological urines other than those containing sugar and albumin, the same tendency to maintain a constant proportion between the freezing point and specific gravity may be observed, although within rather wider limits than in the case of normal urine. The average weight of the molecule or ion in urine may be determined from these figures as approximately 56 to 60, making use of Beckmann's formula,  $M = E^m$ , where  $M$  = molecular weight of the substance dissolved;  $E$  = a constant having the value of 18.5 when water is employed as the solvent;  $m$  = the quantity of substance dissolved, assumed in this case to be 2.33 grams of substance for each 0.01 of specific gravity; and  $\Delta$  = the observed depression of the freezing point ( $0.75^\circ$  for each 0.01 of specific gravity). In diabetic urines, the quantity of sugar may be very readily estimated by determining the lowering of the freezing point of the urine in question, and calculating the theoretical lowering of the freezing point from the specific gravity. The difference between these two quantities in degrees C., when multiplied by 6, gives a very close approximation of the actual percentage of sugar present in the solution. This factor, 6, calculated on a theoretical basis and subsequently confirmed in practice, is dependent on the fact that the molecular weight of sugar is 180, whilst the average of urine solids is below 60. The quantity of albumin may be estimated by determining the specific gravity and freezing point in a urine to which a couple of drops of dilute acetic acid has been added, then boiling in order to precipitate the albumin, filtering and once more determining the specific gravity and freezing point. The second specific gravity is reduced to the same ionic concentration as the first, by multiplying its decimal portion by the freezing point before boiling, and dividing by the freezing point obtained after boiling. This calculated specific gravity is then subtracted from the original specific gravity determined before boiling, and the difference multiplied by the factor 400, which gives the percentage of albumin. In a urine containing both albumin and sugar the former is

first estimated by addition of acetic acid, as above, and the freezing point and specific gravity obtained after boiling are employed in the estimation of sugar.

R. P.

**Bürker, K.** Eine einfache Methode zur Gewinnung von Blutplättchen. *Centralbl. f. Physiol.* **17**: 137, 138, 1903.

No satisfactory method of getting blood plates in large numbers, and unmixed with either white or red corpuscles, has

hitherto been described. Bürker states that the following simple procedure works very satisfactorily. The drop of blood is obtained by puncturing with a lancet or better with a Fancke's needle the skin on the tip of the finger, which has previously been thoroughly cleaned with ether-alcohol. The drop is allowed to fall through as short a distance as possible onto a smooth piece of hard paraffin, or a slide coated with paraffin. The blood drop will not spread on the paraffin, but takes as nearly as possible a spherical form on account of its surface tension. The paraffin plate bearing the blood drop is placed at once in a moist chamber, in order to prevent clotting. There immediately begins in the drop a natural separation of the elements in the blood, on account of their different weights. The red and white corpuscles sink to the bottom, while the blood plates, being specifically the lightest elements, rise to the top. If, after from 20 to 30 minutes, a perfectly clean cover-slip be barely touched to the uppermost surface of the drop and at once raised again, it will bring away a small drop of serum containing a large number of blood plates, and but very few corpuscles. The cover is at once placed on a slide for examination. Clotting begins very soon on the slide and fibrin threads may be seen in process of formation. R. P.

**Moore, Anne.** Some Facts concerning Geotropic Gatherings of *Paramecia*. *Amer. Jour. Physiol.* **9**: 238-244, 1903.

Miss Moore finds that positive geotropism may be induced in *Paramecia* by (a) Mechanical shock. The effect of

shock takes place quickly and is lost quickly. (b) Reduction of temperature. At 1° C. the effect is marked, and takes place quickly. In a comparatively short time, the animals may adapt themselves to the low temperature and lose the sense of geotropism. (c) Increase in concentration of the surrounding medium. This factor is not so constant as the other factors, and the effect not so marked. (d) Lack of food. The effect takes place slowly and is lost slowly. Negative geotropism may be induced by a plentiful food supply and by an increase in temperature within limits. It is pointed out that these reactions may be of some importance in the life history of the organisms. R. P.

**Kreuzfuchs, S.** Die Grösse der Oberfläche des Kleinhirns. *Arb. a. d. neurol. Inst. zu Wien.* **9**: 274, 1903. *Ref. Centralbl. f. Physiol.* **17**: 110, 1903.

The surface area of the cerebellum has not up to this time been exactly determined, and the work of Kreuzfuchs makes a noteworthy contribution in this

direction. In making the determination the vermis and hemispheres of a cerebellum were separated, and serial sections of the three parts were made. The sections were from 200 to 300 mikrons in thickness. The sections were put into anilin oil, projected with an Edinger drawing apparatus, and the projected peripheral outlines measured with a map-measurer ("Curveometer"). As all the sections were made as nearly as possible at right angles to the surface of the

gyri, the product of the length of the periphery by the thickness of the section gave the area of that portion of the surface of the cerebellum included in each individual section. Measured in this way the total surface area of the cerebellum studied was found to be 84,246 sq. mm. Of this total area 16,344 sq. mm. were included in the free surface area, and 67,902 sq. mm. in the surfaces of the fissures. The relation of exposed and covered surfaces is not the same in all parts of the cerebellum. While the cerebrum is from 8 to 9 times heavier than the cerebellum, its surface area is only from 2.2 to 2.6 times greater. The estimated number of Purkinje cells in the total surface area of the cerebellum studied was 14,237,674.

R. P.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn, Wesleyan University, Middletown, Conn.

**Parker, Beyer, and Pothier.** Yellow Fever  
Inst. Bul. 13 of the Marine Hospital Service.

The rapidity with which the etiology of yellow fever has been solved since the close of the Spanish-American war is remarkable. Following close upon the discovery of the mosquito as the means of conveying the disease comes the work of the authors of this bulletin, who have discovered what appears to be the cause of the disease. According to them the yellow fever is caused by an animal parasite, probably a sporozoan, that undergoes part of its life history in the mosquito. Their evidence is not yet complete for they have hitherto failed to find anything in the blood of the human patient that can be regarded as representing the organism. But they find it in the body of mosquitoes that have fed upon the blood of patients, prove that these mosquitoes can give the disease by biting, show that other mosquitoes besides the *Stegomyia* do not contain the parasite and that individuals of *Stegomyia* that have not fed upon yellow fever blood do not contain the parasite or convey the disease, a series of facts that renders the causal connection well nigh demonstrated.

The methods which the authors adopt for studying the mosquito may be useful for all students who are working upon the relation of mosquitos to human diseases. The mosquito is placed alive in a small test tube and either killed with tobacco smoke, or the test tube is simply inverted over a bottle of absolute alcohol and the mosquito allowed to fall into it. After two hours in the alcohol it is transferred to a watchglass, the legs pulled off and the wings cut close to the body, and then the body is transferred to a change of absolute alcohol. After two hours the insect is transferred to xylol and in half an hour placed in the warm chamber of the paraffin bath. Here liquid paraffin is added, a few drops at a time, until saturation, and then the insect transferred to melted paraffin (52°). After an hour it is embedded. Serial sections are cut, usually 15 micra thick. The ribbons are floated by hot water on to a slide, flattened by



rice paper moistened with 80 per cent. alcohol and thoroughly dried. The staining is with iron hæmatoxylin and a saturated solution of bismarck brown in 60 per cent. alcohol.

H. W. C.

**Rosenau.** The Bacteriological Impurities of Vaccine Virus. Bul. 12 Public Health and Marine Hospital Service, 1903.

The use of glycerine in the preparation of vaccine virus has been widely heralded as a means of reducing or destroy-

ing the bacteria in the virus so as to prevent the unfortunate cases of tetanus, blood poisoning, etc., which occasionally follow vaccination. Much interest has therefore attached to the question of the presence of bacteria in vaccine and the value of glycerine in destroying them. Rosenau has made a long series of bacteriological examinations of virus to answer these questions. His method consists in placing the virus in a measured quantity of sterile bouillon, agitating until all clumps were thoroughly broken up. The dry points were first softened in a small quantity of bouillon and then rubbed clean in bouillon with proper precautions. The glycerinated virus from capillary tubes was also mixed with bouillon and the capillary tube washed clean. Especial care was necessary to insure the breaking up of the clumps. From the bouillon mixture agar plates were made in which a definite number of drops of the bouillon was mixed. The plates were incubated at 37° and after growth an estimation of the total number of bacteria in each dry point or glycerine tube was easily obtained. The result of the work in brief is to show that no form of virus is free from bacteria although the glycerineated virus is purer than the ordinary dry points.

H. W. C.

**Muller.** Ueber das Wachstum und die Lebensfähigkeit von Bakterien, sowie den Ablauf fermentativer Prozesse bei niedriger Temperatur unter spezieller Berücksichtigung der Fleisches als Nahrungsmittel. Arch. f. Hyg., XLVII, p. 127, 1903.

The interest that has developed in recent years concerning fermentative changes at low temperatures, especially in reference to ripening of cheese, makes this investigation of Muller of particular

interest. Most of the work reported has been carried on at a temperature of exactly 0° C. For maintaining such a temperature the author devises an apparatus with three chambers inside of each other. The material to be tested is placed in the inner one and the other two are filled with ice. The ice in the outer chamber is changed as it melts, while that in the middle chamber melts very slowly and needs little change. A thermometer shows that a constant temperature of freezing is easily maintained for a long time. With such a device he tests the growth of several bacteria and the fermentative power of several enzymes. His general conclusion is that bacteria grow readily enough at freezing, but, of course, much more slowly than at higher temperatures. The fermentative action of enzymes also continues actively at these temperatures. The author also tests the action of temperatures below freezing. These lower temperatures, however, he is not able to control so closely, and the results are not so exact. He finds that bacteria do not grow at temperatures below freezing. The curing or ripening of flesh, which is known to go on at low temperatures, even below freezing, Muller regards as due chiefly to the enzymes present rather than to the action of bacteria.

H. W. C.

## GENERAL LABORATORY TECHNIQUE.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoological Laboratory,  
University of Michigan, Ann Arbor, Mich.

### Model of Cerebral Ventricles.

In the *Jour. Anat. and Physiol.*, Vol. XXXVI, Part 2, 1902, pp. 106-126, Barratt describes the making of a model of the human cerebral ventricular cavities. A brain was selected which showed but slight evidence of wasting, and in which the cerebral ventricles contained a small amount of fluid. The brain was carefully removed and placed at once, without being incised or the membranes stripped, in a saturated solution of potassium bichromate, in which it at first floated, slowly sinking at the end of one or two days, but remaining of nearly the same specific gravity as the bichromate solution, for a length of time sufficient to allow a well defined hardening of the surface to occur. By this method of hardening a minimum change of form is induced. After hardening was completed, sketches of the outline of the brain and its principal sulci were made. The brain was then sectioned in a vertical transverse plane at intervals of  $12\frac{1}{2}$  mm. The slices as they were cut were placed in bichromate solution, and a tracing of each was made without delay. In order to be able to reconstruct the ventricles from the sections it was necessary to have two planes marked out on each section, one horizontal and one sagittal. The former was obtained by placing the brain before sectioning in a frame by the aid of which an incision was made all around its outer surface in a horizontal plane. The second was the mesial plane of the brain, indicated by the great longitudinal fissure and the structures in the middle line at the base of the brain. A plan and front and side elevations of the ventricles were then made and copied onto a block of wood. By this aid and with the sketches a carving of the ventricular cavities was then made. From the figures given it would appear that the procedure followed was very successful.

R. P.

### Photographing Living Infusoria.

In *Biometrika*, Vol. I, Part 4, p. 401, 1902, Simpson describes a method which he has used in obtaining photographs of living ciliates. As apparently such photographs have not been made by other workers, it may be of interest to JOURNAL readers to know the procedure followed. Simpson says: "Ordinary Ilford chromatic plates were used, as also a Leitz 3 lens, which with the associated extension gave a magnification of about 80. The pictures were all taken instantaneously by incandescent light. The proportion of failures was very high, about 70 per cent. The chief difficulty was in obtaining a cell small enough to be wholly included within the magnification of the lens. Ultimately a block of soft paraffin was employed in which a hole was pierced with a fine needle. It was then sectioned with the microtome, and in this way by regulating the thinness of the section, a cell was obtained with a minimum of water in which the infusorian could live and yet be in focus all the time. A cover-glass was then superimposed, and as it was held in position by a generous application of vaseline round the edge, I was able to take photographs in a horizontal position. Some of the photographs were sadly lacking in definition, but it is a matter of extreme difficulty to calculate and adapt that amount of water in the cell which is sufficient for the free movement and life of the protozoan and yet is not too great to allow it to get out of focus during the exposure."

R. P.

# Journal of Applied Microscopy and Laboratory Methods

**Vol. VI.**

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# Journal of Applied Microscopy and Laboratory Methods

VOLUME VI.

OCTOBER, 1903.

NUMBER 10.

## Laboratory Work in High School Physiology.<sup>1</sup>

"Try any plan that you wish," said the wisest high school principal I have ever known, "Try any plan that you wish; I have never yet seen a course in physiology that amounted to anything." This was an epitome of the situation in New York city seven years ago when the high schools were opened, and I have come to learn that this feeling in regard to physiology courses is the common one, held by a large number of leading educators.

Now, surely this state of things ought not so to be. It certainly is not difficult to interest a boy in the wonderful processes that go on in his own body, and to lead him to an inductive study of these processes. For his own comfort and happiness it is essential that a youth become an intelligent keeper of the body committed to his care. And if we are to develop an intelligent and lasting public spirit in regard to the matter of home and city sanitation, we must teach the necessary lessons to boys and girls at an age when lasting impressions can be made. Physiology, then, should be regarded as a worthy subject of study, both from its educational value and from its practical bearings on the life of the individual, the home, and the community.

But why such a widespread contempt for this subject? While there may be other reasons, I venture to suggest the three following:

In the first place, in most elementary schools and in a large number of high schools, physiology has not been taught from the standpoint of *function*. Boys and girls are intensely interested in the uses or adaptations of things. But instead of teaching them physiology, by which we mean the uses or functions of the various organs, we have been compelling them to learn either tiresome anatomical details or dry-as-dust rules of hygiene. They have asked us the uses or whys of things, and we have given them instead the names of a lot of bones and muscles, or a series of thou shaltst and thou shalt notst.

In the second place, there are probably fewer specialists who are engaged in the teaching of physiology than is the case in any other subject. You often hear of music teachers and teachers of Latin who are assigned the extra classes in physiology, because, forsooth, we must in every school at least go through the form of hearing recitations on the human body. Uninteresting text-book

<sup>1</sup>Address before Science Section of National Education Association, Boston, July 9, 1903.

lessons are therefore inevitably the result, and pupil and teacher alike come to detest the subject.

Why, then, has not the teaching of physiology been consigned to the rubbish heap with other educational ideas that have been found to be impracticable or useless? The answer to this question is perfectly simple. In every state of the Union but two, statute laws threaten with more or less dire penalties school authorities who do not devote a disproportionate amount of time to instruction as to the effects of alcohol and narcotics. No other subject, so far as I know, is thus dominated by legislative enactment. The result is that our high school pupils, at least in New York, call their physiologies "the liquor books," and while able to tell you a good deal about delirium tremens, are utterly ignorant of the commonest processes involved in nutrition.

How can we expect satisfactory results either from an educational or practical point of view, from the study of physiology when the method of approach is wrong, when the subject is taught by untrained teachers, and when most physiological instruction is avowedly an appendix to instruction on alcohol and narcotics?

So much for the past, and possibly for the present situation. Let us now look at the possibilities which lie open before the well-trained and enthusiastic teacher of physiology. At the outset it is important to emphasize the fact that physiological processes can never begin to be understood, unless the pupil is given some idea of at least the simpler principles of chemistry. He must become more or less familiar with carbon, hydrogen, oxygen, and nitrogen; he must know how to test for carbon dioxide, for acids, and alkalis, and he must learn something of the common processes of oxidation, neutralization, and evaporation. Unless these lessons are taught early in the course and taught by experiment, the boy will find his foundation weak when he attacks the more difficult processes involved in digestion, respiration, and excretion. Most of these simple lessons can be taught from common matches, a few chemicals, and pieces of glassware. If a pupil once gets clearly in his mind the nature of elements, compounds, and the process of oxidation, an immense amount of subsequent labor and disappointment will be saved.

Suppose now we take up the important subject of foods. The pupil should first familiarize himself by laboratory experiments with the five or six nutrients that are found most commonly in foods. For the starch tests a quart of iodine solution can be made for a small sum, and this, put into small bottles, will supply a large class. Most of the experiments in food analysis can be carried on successfully at home, the only real difficulty being that the pupil is likely to get so interested in his experiments that he forgets to learn his other lessons. When he has followed the simple directions given him, has tested ten to twenty foods, and has been called upon to defend his results in the class room, a boy is not likely to forget that cereals usually contain a large amount of starch, and that this nutrient is absent in foods of animal origin. Instead of relying on text-book authority, he has demonstrated beyond a doubt that potatoes and flour contain a large quantity of starch, and that peas and beans commonly have less of this nutrient. The conflicting results derived from the testing of spices furnish a good text for a discussion of food adulterations.

The Fehling's solution test for grape sugar can be performed at home, the necessary chemicals and test tubes being furnished the pupil. The presence or absence of proteids, fats, mineral matters, and water should likewise be experimentally determined at home or in the class by the individual pupil, and a comparison made of the results obtained. Each pupil has now a concrete idea of some of the most important compounds he is to meet continually as ingredients of his food, as components of his blood, or as essential constituents of his body.

It is impossible of course to demonstrate by experiment the uses of the various nutrients, and so with laboratory study there must be combined a considerable amount of class room recitation. Indeed, we should bear in mind that laboratory work must always be followed with and supplemented by vigorous questioning; a process which keeps clear in the mind of the pupil the essential points in each experiment and the relations of the various facts that have been learned.

The uses of foods, proper methods of cooking, and the study of food economy are, to my mind, among the most important of the topics connected with human physiology. These subjects are treated more or less inadequately in most school text-books, but fortunately the publications of the United States Department of Agriculture are available even in the large quantities required for individual study. The best bulletins for high school use are "Foods: Nutritive Value and Cost," and "Meats: Composition and Cooking." The colored food charts which are so useful in class recitations are unfortunately out of print, but the charts and tables in the bulletins named above can be used to almost as good a purpose.

A study of half a dozen of the common tissues should next be undertaken, because these tissues are met with again and again in considering the processes of digestion, circulation, and respiration. Get a butcher to saw in halves the leg bones of a sheep, supply each pupil with a half bone, and a dissecting knife. At the close of fifty minutes of laboratory work, he should have learned the essential characteristics of bone, cartilage, connective tissue, and fatty tissue. Another laboratory period spent in studying pieces of beefsteak will fix in mind muscle tissue and will serve to review fat and connective tissue. It is perhaps best to reserve the discussion of glandular tissue, nerve tissue, and respiratory tissue until a later time.

The study of cell structure and cell functions should now be introduced, and two or three days may well be spent at this time on *Amœba* and *Paramecium*. The pupil should be led to see that these single celled animals carry on processes essentially the same in kind as those performed by the highest animals, and it is well perhaps to consider, in the following order, the ten most important functions carried on by animal cells: (1) locomotion, (2) taking in of food, (3) digestion, (4) circulation, (5) assimilation, (6) taking in of oxygen, (7) oxidation or metabolism, (8) excretion, (9) sensation, and (10) reproduction.

Let me take a little time to suggest some methods that have been found effective in this laboratory study in the classes of a large school. While it is not impossible to do this kind of work even when the teacher has to go from room

to room, school principals should be led to see the enormous advantage, both to pupil and teacher, of the single laboratory. The room used for this subject need not be equipped with special furniture, for ordinary desks will answer almost as well for most of the work in physiology. One essential condition, however, is an adequate supply of fresh material, which can be furnished by any local butcher who has access to a slaughter house. These supplies are, of course, to be paid for out of public funds, just as truly as are text-books and chemicals. After the laboratory has once been equipped with permanent apparatus, the total expense of the course ought not to aggregate more than 8 to 10 cents per pupil.

Suppose our topic of study is muscle, and we are aiming to lead the pupils to get at first hand clear ideas of its structure. Pieces about the size of one's thumb, cut from the leg muscle of a cow, should be placed on each desk, together with a dissecting needle. In small classes it may be best to allow each pupil to discover what he can and to report his observations orally. But in divisions of forty this method of procedure is likely to result in desultory observations from the few and inattention and mischief on the part of the many. Instead, let each pupil receive a mimeographed or printed set of questions something like the following :

1. Pull apart more or less the small *bundles* of which the meat is composed. What is their shape? Are they all of the same size?

2. What is the color of the meat? What do you infer as to the presence or absence of blood?

3. What are the characteristics of the tissue (called perimysium) which surrounds and connects the muscle bundles?

4. Can you distinguish any fat in the piece of meat that you are studying? Make sure of your answer by rubbing the meat on a piece of paper. If fat is present, where is it situated and what are its characteristics?

5. Is tendon (known also as gristle) present? If so, give some of its characteristics.

6. Make a drawing of the piece of muscle (magnified about three times) showing cross and longitudinal sections. Label bundles, perimysium, and fat and tendons if they are present. See "Laboratory Exercises in Anatomy and Physiology," Henry Holt & Co., New York City

Every pupil is at once busy writing down in complete sentences the results of his observations. Meanwhile by the use of three or four compound microscopes the pupils in turn may be shown the microscopic structure of a bit of beefsteak, and when they have taken their seats, they are prepared to write the answers to two more questions, namely :

1. Of what is the piece of muscle found to consist?

2. Why is this kind of muscle called *striped muscle*?

A text-book lesson on the structure of muscle can now be learned in half the time and with twice the interest. The papers of the previous day should then be handed back and the necessary corrections should be made by the individual pupil. Having now seen muscle tissue, discussed its structure, and studied the descriptions and pictures in the text-book, stupid indeed must be the boy who cannot give you a year or two later an intelligent account of muscle.



Let us now see how much we can learn of the anatomy and physiology of the human arm and hand by simple observation and experiment. One can easily feel through the flesh and determine that the skeleton of the upper arm is formed of one long bone which has an enlargement at either end. The two bones of the forearm can also be distinguished and described. We cannot of course count the small bones of the wrist, but the range of motion between them can be shown easily. In the palm and fingers the position and shape of the bones can be determined almost as well as by studying a prepared skeleton. Having learned all that is possible from his own arm, the pupil's attention should be directed to a study of an articulated skeleton. And before any text-book lesson is assigned on a given set of bones, the bones should be pointed out by the pupil on his own body, or on the skeleton, and their form and use studied.

The position and action of the muscles of the arm are even more easily determined. Why should a boy learn from a book that his biceps muscle on contracting becomes harder, thicker, and shorter, when by a few laboratory directions he can so clearly demonstrate this fact? He can make out, too, that the lower tendon of this muscle is attached in the region of the forearm, and after grasping this tendon between the thumb and forefinger, he can by turning the forearm satisfy himself that this muscle is joined to the radius and not to the ulna. A similar study of the fleshy part of the forearm reveals to the pupil that the muscles of the fingers are located in this region. By closing and opening the fingers the long tendons which pull on the fingers can be traced from the forearm, along the wrist and the palm or back of the hand to the bones in the digits. One can easily see how clumsy would be the hand were all the flexor muscles of the fingers located in the palm, as the flexors of the thumb are.

The essential points in the structure of the skin, of the nails and the hair, of the action of the blood-vessels, sweat glands, and nerves in the hand can also be found out by the individual pupil. It may seem to take a bit longer to acquire these facts by observation than from text-book lessons. But that the laboratory method of study is far the better is clear from the interest manifested by the pupil in carrying on his work, from the greater clearness with which he can describe what he has learned, and from the fact that he can in a moment's time, even when writing an examination paper, test the accuracy of his statements by a repetition of his original observations. It is amusing at times to watch a class while taking its final test in physiology. If anyone who had not watched the laboratory work through the term should enter the examination room, he would be at a loss to interpret the apparent contortions of the muscles, joints, and other organs of the pupil's body.

There is no time to suggest some of the interesting and instructive laboratory experiments which can be used to make clear the processes of digestion, circulation, respiration, and sensation. When possible, these experiments should be performed by each pupil on his own body. If models and pieces of apparatus are to be used instead, they should be as simple in their action as possible, and attention should always be called to the fact that no model can be made to work exactly like the living organs and tissues.

We have now briefly discussed some of the experimental work that is possible

in the study of the human body itself; let us look for a moment at a few of the related topics that may be studied to good advantage in a course like this. Boys and girls are by instinct comparative anatomists and physiologists, and the study of the human body offers rare opportunities *to develop* this instinct. Suppose your class has been studying the skeleton. If there is a natural history museum within an hour's ride, take them there; give them a set of questions which will apply to the skeleton of any vertebrate, and set them at work on the skeleton of a giraffe, a horse, or an elephant. Unless your experience is exceptional, you will find an excited group of boys and girls plying the skeletons and you with questions as to the position and use in the specimen before them of the various bones which they have studied in class.

Teeth, too, are wonderfully interesting when studied comparatively and in relation to the food which the animal eats. The school museum should contain at least the skull of a horse, a dog, and a rat or a squirrel, for these can easily be procured by the teachers or pupils. These are common animals with which every boy is familiar, and he is thoroughly interested in making a study of the machinery by which these animals grind, tear, or gnaw their food. Other profitable subjects for comparative study are the various methods of locomotion employed by vertebrates and invertebrates, their methods of getting their food, the ways in which they are protected, and the sense organs which they possess. Much of this observation can be done at home, or afield, or in zoölogy parks by the individual pupil, if once he acquires the habit of noting resemblances and differences.

Before closing just a word or two in regard to the study of bacteria. If you wish to teach cleanliness most effectively, devote a half dozen lessons to the study of these micro-organisms. Let the pupils experiment at home with milk and with a hay infusion. Expose culture dishes containing nutrient agar to the air in the corridors before and after sweeping, and let the pupils note the growth of the colonies of bacteria day by day. Teach the boys and girls the principles of inoculation and sterilization, and show them with high power lenses the living germs under the microscope. Emphasize the filthiness and the danger of expectoration (better call it spitting) in public places, and call attention to the splendid work done by boards of health and by such men as our New York Waring and Woodbury. We physiology teachers ought to win thousands of votes each year for a clean city government.

But you will doubtless say it is impossible to consider all these topics in the time assigned to this subject. Perhaps the New York authorities have been rather more generous than those of other cities. Biology is a required subject for all classes throughout the first year, five periods per week. The first half year is devoted to the study of botany; and in the second twenty weeks there is time for all the work suggested in this paper and for a great deal more.

Physiology, then, need not be uninteresting and unprofitable. If taught by laboratory methods, it is replete with interest. From an educational point of view it well deserves consideration as an inductive science, and in its practical bearings it is even more useful than the other sciences which are now honored in the school curriculum. As physiology teachers, however, we have much to do along the lines of choosing our subject matter and of developing our pedagogics. When we have done this and are able to point to years of successful experience, this subject in which we are interested will be given the place in the school curriculum which it so richly deserves.

JAMES E. PEABODY.

Morris High School, New York.

## Microscopical Work in Turkey.<sup>1</sup>

The following is not intended to be a concise report on the condition of microscopy in the Turkish Empire, but only a general sketch of what the most important lines are along which work is done.

There being only one states-university in a country with about 25,000,000 inhabitants, and only seven or eight colleges established by foreign missionary societies, with perhaps about a dozen native institutions of the rank of a college, or rather of a gymnasium (*lycée*), there is certainly not too much of an opportunity for microscopic work being carried on. To undertake preparing a census of microscopists would be groping in the dark, as there is no basis for any calculation whatever. We have to be satisfied with a very approximate estimate of the number of microscopes.

If we consider in the first place the different centers of education, I doubt if an average of five microscopes is not too high a number for the 20 to 25 higher institutions, even including the three or four medical departments. I know several institutions which have only one or two, and know only one which has about forty microscopes.

The prospect becomes brighter when you take into consideration the educated professionists, as physicians and druggists, especially such as have had their training wholly or in part in Europe or in America. Among this class of people there may be probably a total of 1000 or more instruments in use. A great number of doubtful diagnoses are settled by the penetrating vision of the microscope, many a criminal case is brought before its court of impartial judgment, and disputes between a custom-house officer, demanding exorbitant duty on some article, and the niggardly owner who wants to escape paying by inventing some fictitious name for it, are referred to the just and true decision of the microscope.

How many of the microscopes falling under the categories mentioned may be in active use, and how many may be spending a life of laziness on dusty shelves or in dark cases, it is hard to tell, but that the discoveries made by the aid of either of them would not fill volumes, is certain.

With my own classes in biology in our college I have always found a great desire for looking at microscopic objects, but as during the thirteen years of teaching I have met only a few students who desired to make personal investigations, it seems that it is only curiosity which guides them. This being once satisfied the volunteers gradually disappear, until at the close of the term you find yourself left with one or two attendants, if not quite alone. Yet it would not be fair to throw all the blame on the students, as the full program of lessons, together with the necessity of acquiring four different languages (Turkish, English, French and Vernacular) leaves them very little leisure to follow a special line, requiring continuous effort and attention.

Notwithstanding all less favorable circumstances already mentioned, there is

<sup>1</sup> I wish to express my very special gratitude to Mr. H. K. Pepeian of Amassia, for furnishing notes on the use of the microscope in silk business.

J. J. M.

one department in which a great deal of microscopic work is done in a very practical way. This is silk raising. The scientist of immortal fame, Pasteur, has exerted his influence far beyond the limits of his native country, and though he himself, as far as I know, has not accumulated riches, he has been to many the guiding star to the Golconda, if not of jewels, yet of equally precious silk. The discovery of Pebrina, Mycosis and other diseases of the silkworm, and especially the exposition of the fact that the germs of these diseases can be detected in the bowels of the moth, have pointed out a way how to raise sound eggs, from which a strong and healthy breed may be expected, provided proper care is taken in rearing them.

This was a new field for microscopists. France soon became the center of a lucrative trade in silkworm eggs, which were soon imported into Turkey. They were brought in round pasteboard boxes, 10.5 cm. in diameter, and 2.5 cm. high, perforated with rows of little holes to allow for the necessary respiration of the sleeping germs, and containing about 25 gm. of eggs. Fifteen years ago such a box used to sell for about six to eight dollars, but this did not last very long. The Imperial Turkish Government quickly recognized the promising future of this new discovery and, to utilize it, a school for silkworm culture (*séri culture*) was founded at Broussa (at the base of the Asiatic Olympus). Here in a year's course all that is known about the various diseases of the silkworm, their diagnosis, their treatment and more especially their prevention, is taught or explained by aid of the microscope. For their prevention hygienic principles are laid down and are always crowned with success if strictly followed. Study is connected with practical work in the nurseries as well as in the mulberry plantations. Prizes are awarded to those students who show marked ability and progress, microscopes being mostly used for that purpose. This institution is now sending out annually 30 students, the number being limited by law. There are alone in Broussa about 400 graduates of this school and their assistants, constantly busy with their microscopes, and the whole number of instruments in use in this line in the whole country may be about 4000. The chief centers are Broussa with its environments, Beyrout with the Lebanon, Amassia, Smyrna, Harpoot and Antioch. Secondary places are numerous.

The evolution of the whole business has been astounding. Mulberry plantations had been low in value on account of the gradual deterioration of the silk business, but now they represent 4 to 5 times their value at 1876. The production of raw cocoons was about that time in Amassia only 10,000 Ko. a year, now it averages 130,000 Ko., and in the district of Broussa it rose from 300,000 to 5,000,000 Ko. a year. (It should be remembered that only one-ninth of this quantity will be finally put into use as finished silk, as the pupa in the cocoon constitutes the main bulk of its weight, and the cocoon itself is moist while fresh.) The annual output of the whole country is now about 600,000 Ko. of silk, corresponding to about 5,400,000 of fresh cocoons, but as there is a great deal used up for home manufacture, the total is probably 20 to 25 per cent. more.

Besides the direct raising of silk there is much business carried on in the sale of the eggs (called vulgarly silk-seed), of which one to one and a half million



boxes at 25 gm., are now exported every year to Caucasia, Persia and Turkestan, at a price of about 60 to 80 cents a box.

The government has this year started a competition in the silk business, awarding prizes in the form of microscopes or cash amounts, for proficiency in (1) Egg-production, (2) Cocoons, (3) Nurseries, (4) Plantations. Nine chief centers are selected, and in a cycle of three years three places will be visited every year by special officers and about \$660 will be distributed at each place in prizes.

The microscopes in use in the whole business are mostly from Nacet & Fils, Paris, or from F. Koristka, Milan. The magnifying power generally used is 500 to 700 diameters.

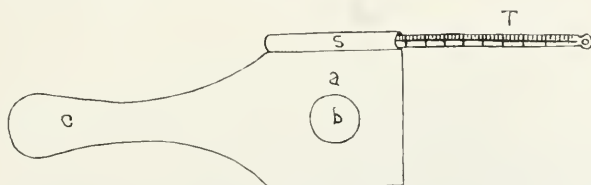
I need dwell no longer on the great benefit accruing to this country from a single discovery. May there be many other similar discoveries made by the bright minds all the world over.

J. J. MANISSADJAM.

Anatolia College; Merzifun (Marsovan), Turkey in Asia.

## A Simple Thermometer Attachment for the Copper Plate Warm Stage.

A copper plate with a projecting arm heated by an alcohol lamp or a gas burner is well known as a rough and ready warm stage for the microscope, but the following simple thermometer attachment to such a stage is, so far as I am aware, new and furnishes a means of determining and regulating the temperature. This attachment consists, as the accompanying figure shows, of a sleeve (s) for receiving an ordinary chemical thermometer (T), formed from an edge of the stage part of the plate (a) bent into a tube. A warm stage with this attachment can be constructed very easily from a piece of sheet copper. The side of the stage



to form the thermometer sleeve should be cut a trifle more than three times the diameter of the thermometer longer than the opposite side. This projecting side is then rolled up into a tube.

The thermometer attached in the position shown in the figure balances the arm (c) to which the heat is applied, and is in a position convenient to be read. The bulb of the thermometer being at approximately the same distance from the source of heat as the center of the stage and entirely surrounded by the copper plate, the temperature registered will conform closely to that of the center of the stage.

ERNEST LINWOOD WALKER.

Massachusetts State Board of Health.

### The Ohio Lake Laboratory.

The opening of the new Lake Laboratory on Cedar Point at Sandusky, Ohio, July 2, 1903, makes a new epoch in the development of biological science in the Middle West.



FIG. 1.—Near view of laboratory.

The rapid development of the biological sciences has resulted in the organization of a number of marine and inland laboratories. Among the earliest of these laboratories was the Lake Laboratory at Sandusky, Ohio. The first work



FIG. 2.—Laboratory from the beach.

in this region looking towards the organization of a summer laboratory was done by Prof. D. S. Kellicott of the department of zoölogy of the Ohio State Univer-

sity. In 1896 a second story, built especially for laboratory purposes, was added to the Ohio Fish Commission building at Sandusky, and these temporary quarters were used until the present season.

The laboratory remained under the direction of Prof. Kellicott until his death in 1898, when it came under the direction of his successor, Prof. Herbert Osborn, the present director.



FIG. 3.—The cove from the roof of the laboratory.

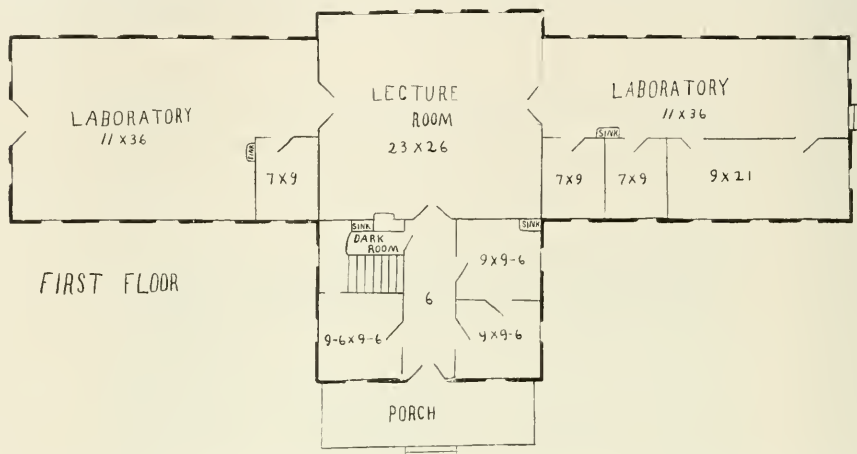
The laboratory was first intended for investigation only, and for the first four seasons was used by only three or four workers. Since that time undergraduate courses have been offered, but research is still the predominating feature of the work.



FIG. 4.—Cedar Point from the roof of the laboratory.

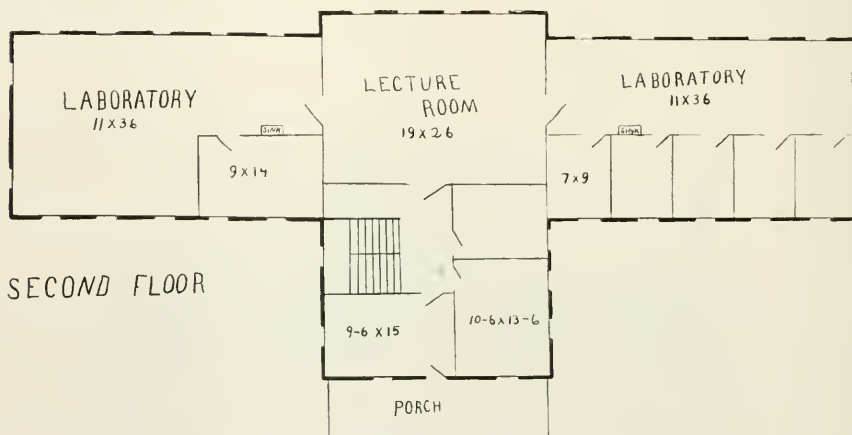
For some time it has been recognized that the quarters in the Fish Commission building were not sufficient and that the location was not at the most satisfactory point on the Sandusky Bay. In 1902 the trustees of the Ohio State University appropriated \$2500 for the erection of a building exclusively for laboratory purposes, to be located on Cedar Point on the opposite side of the bay from the old laboratory. This new building was formally opened July 2, 1903.

The new laboratory is a two-story building, 23 x 98 feet, with front extension of  $18\frac{1}{2}$  x 25 feet. There are two large lecture rooms, four large laboratories, 18 small rooms for investigation, an office and a dark room. The equipment consists of three boats and the necessary dredges for working on the aquatic



fauna and flora. The microscopes, microtomes, other apparatus, and library are supplied from the Ohio State University laboratories.

It would be very difficult to find a region with a richer fauna and flora than the region of the Sandusky Bay. The bay with its many marshes, and the rivers and creeks emptying into it, the open waters of the lake, the many islands, Cedar Point which is really a large sand bar separating the bay from the lake,



and the prairies and forest lands to the south, all present conditions favorable to the development of a rich fauna and flora. The location of the laboratory is at a point easily accessible to collections of all kinds of fresh water and land forms of this latitude. It is very doubtful if it is possible to find a point where the fauna and flora are found in greater variety and abundance, and where they are more accessible than from the Lake Laboratory.



At the formal opening of the laboratory addresses were made by Prof. J. W. Denny, Dean of the College of Arts, Philosophy and Science, O. S. U.; Capt. Alexis Cope, Secretary of the Board of Trustees; John T. Mack, resident trustee; Prof. Herbert Osborn, the director; and Prof. C. J. Herrick, of Denison University, President of the Ohio Academy of Science. MEL T. COOK.

DePauw University, Greencastle, Ind.

## The Technique of Biological Projection and Anesthesia of Animals.

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### XVIII. DIRECTIONS FOR MOUNTING LIVE ORGANISMS IN GLASS CELLS—Continued.

Mounting plant specimens for demonstrating the circulation of protoplasm in their cells.

For this interesting, but somewhat difficult experiment, any one of a number of different species of plants, including *chara*, *nitella*, *tradescantia*, may be used. If *chara* or *nitella* are to be used, select rather young, vigorous leaves from whorls near the apex of the stem and study their terminal cells. Different species of purple *tradescantia*, or spiderwort, have numerous fine hairs attached to each stamen. The cells composing these hairs have transparent walls and the cell contents show a natural differential staining. The streams of finely granular, transparent, or silvery, protoplasm circulate through a purple colored sap.

In mounting specimens for this experiment, great care must be taken that the cells are not killed by rough handling, drying, or pressure of the cover-glass. As soon as it is mounted, examine the specimen under an ordinary compound microscope and note the location of the cells which show the circulation best, and transfer the slide to the projection microscope, which should have the light and approximate focus already adjusted for the work. This use of an ordinary compound microscope with very difficult or delicate objects for locating the parts having special interest saves time and specimens, and enables the operator to know how much is to be expected from an object.

B. Directions for mounting various organisms, e. g., *hydra*, *gammarus*, *daphnia*, *cyclops*, planarian worms, leeches, larvæ of insects, small snails and crayfish, and plant specimens, in hollow-ground cells and ring cells.

In this class of work two difficulties frequently arise at the same instant; a lively animal attempts to escape from the cell and air bubbles enter the cell while the cover-glass is being placed in position; but success is easily attained by the method here given.

Having selected and thoroughly cleaned a cell deep enough to hold the organism (Fig. 9, either No. 2, 3, 5-9, 11-13, 21, or 22), and a cover-glass free from flaws and large enough to considerably more than cover the hollow, *partially* fill the hollow with water and place the organism in it. Lay the cover-

glass over one side of the hollow, Fig. 10, so that the water in the hollow will run in between the cover-glass and slide and the organism will be in the hollow under the cover-glass. Slides may be left in this condition for hours, ready to be closed just before use, if placed under a bell jar in an atmosphere saturated with moisture; but they are usually closed in at once, for the organisms live for surprisingly long periods in the closed cells. To close the cell and exclude all air bubbles, push the cover-glass over the hollow and, *at the same time*, add just enough water from a pipette to exclude all air, and then center the cover over the hollow. Press the cover-glass gently, and any excess of water will appear at its edges and must be removed by absorption or with a pipette, as in mounting amœbæ, etc., on plane slides. When all excess of water has been removed, the cover-glass will adhere to the slide in whatever position it is held, and it is now ready for use on the projection microscope.

After a time, evaporation at the edges of the cover-glass may cause air to be drawn into the hollow. If it is necessary to remove it, place the slide on a table,

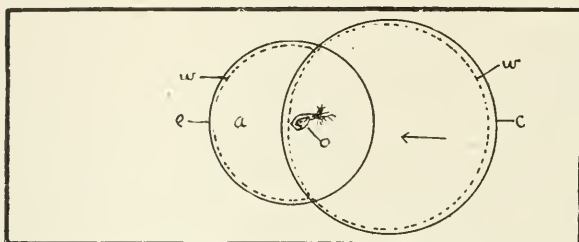


FIG. 10.—Hollow-ground slide with the hollow *a* nearly filled with water *w* containing an organism *o*. The cover-glass *c* is in its first position with water *w* underneath. The cover-glass is to be pushed in the direction of the arrow, while just enough water is being added with a pipette at *e* to exclude air bubbles from the hollow as the cover-glass is pushed over and made concentric with it.

push the cover-glass along until one edge of the hollow is exposed, tilt the slide so that the air bubbles gather at the open edge of the hollow, add enough water with a pipette to expel the air and replace the cover as at first.

C. Mounting organisms, e. g., colonies of *spongilla*, active species of all types, plant specimens, etc., in open top cells.

The use of this type of cells (Fig. 9, No. 14 and 16–20) is so easy that all necessary directions will be included in descriptions of three interesting experiments, the evolution of oxygen by green plants under the action of sunlight or electric arc light, the circulation of water through colonies of *spongilla*, and the study of larvæ and pupæ of the mosquito under hypnosis and anesthesia produced by chloretone.

The demonstration of the evolution of oxygen by green plants is an exceedingly instructive and interesting experiment, especially when the different effects produced by varying the degree of heat and the application of cold are shown in succession on the same specimen. Select several vigorous shoots of the common choke-pondweed (*Anacharis* or *Elodea Canadensis*), a perennial which grows well in aquaria throughout the year, and cut off the stems with scissors about an inch, or less, if the cell to be used is small, from the apex, being careful not to injure

the leaves or stem except at the point of section. Place the tips at once in a glass of water from the aquarium and exhale into the water through a tube several deep breaths. This operation charges the water with an excess of carbon dioxide, which is absorbed by the plant cells. Keep the specimens away from direct sunlight or arc light until ready for use. To mount them, half fill a cell, No. 17 is best, with water from the glass and place in it one or two of the tips *with their cut ends up and fully submerged*, so that the bubbles of oxygen may be seen as they increase in size at the cut end of the stem and become detached and rise to the surface. Focus on the cut end of the stem first, and note the absence of oxygen bubbles, or the slow rate at which they appear. Move the slide on the stage and study the leaf arrangement, form, and cellular structure while the light is acting on the plant. Again focus on the cut end of the stem. To increase the activity of the cells remove the water tank used for cooling the light, so that the full heat power may act. To imitate the conditions of a frost, put a pipetteful, or less, of ice water in the cell while it is under observation, and note the marked decrease in the evolution of oxygen. Remove some of the water with a pipette and drop into the cell a small sliver of ice, which, if not too small, stops the evolution of oxygen and kills the protoplasm of the cells so that activity can not be renewed. While the ice is melting, convection currents of cold water may be seen falling through the warmer water in the cell. A. H. COLE.

## The Museum.

### IX.

#### THE CASE—Continued.

Relief maps, geological features, as mud flowage, tracks, ripple marks, etc., can be framed in low, flat cases on legs, glass tops and sides (Fig. 68), or simply framed, face exposed, and fastened to walls, or left on the floor on rollers (Fig.

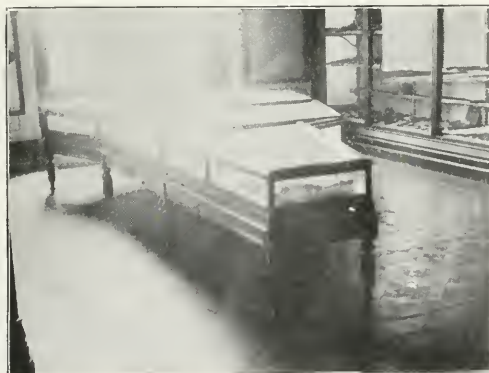


FIG. 68.—Relief map in floor case on legs.

69), or placed on wooden pedestals. The material for all cases should be wood in the frames; black walnut, ash, chestnut, oak, or mahogany, or, in case of nec-



FIG. 69.—Example of large wooden base.

essary economy, pine, stained, and the glass should be plate, American or French. Or all of these mahogany, or the Honduras Bay wood, forms the most beautiful material, its rich and durable tint affording the most attractive color effects. Rose wood can be used with great elegance in special cases, but of course exacts some unusual concessions from the treasury. Ebonized wood has been

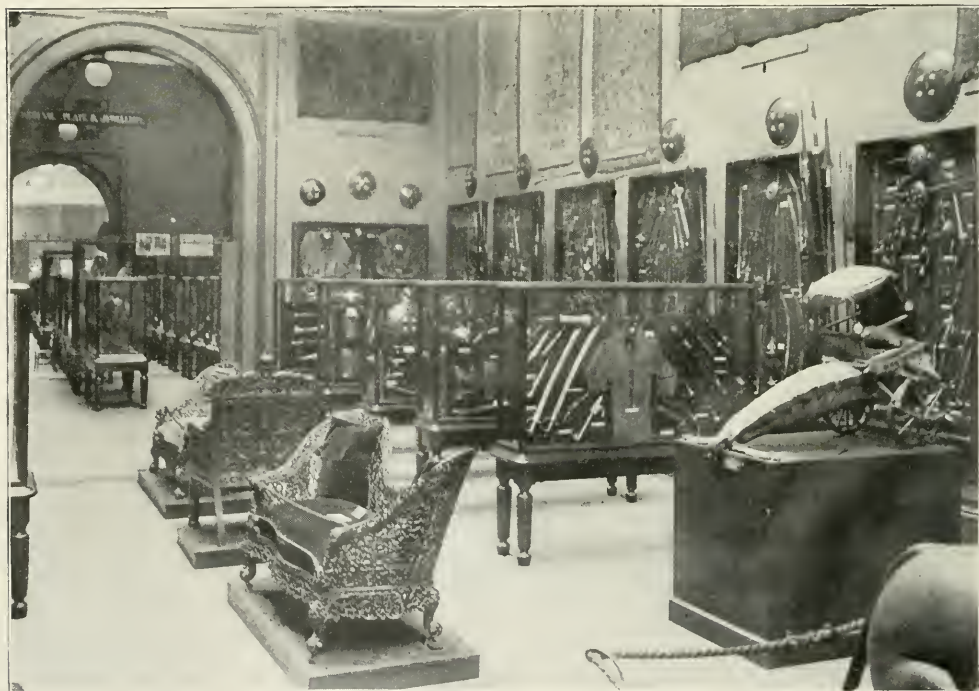


FIG. 70.—Ebonized wood cases, Victoria and Albert Museum, London.



adopted with most chaste effect in the Metropolitan Art Museum in New York. Certainly the halls where the Cypriote statuary is installed, in these dull surfaced and well designed cases, present most pleasing museum pictures. Figure 70 shows a hall in the Victoria and Albert Museum, London, where the wood work is of ebonized wood, and where the armor and implements are artistically assembled. Ebonized wood has been used in the gem room of the New York Museum, and the nature of an octagon case and its surrounding wall cases, in this instance, will be shown in the chapter on Accessories.

Dr. A. B. Meyer of the Museum in Dresden has pressed the claims of iron in the construction of the furniture of a museum on the grounds, primarily, of its safety, and not less indeed on account of its superior beauty. In positions exposed to dangers from fire, for the preservation of books, records, archives,

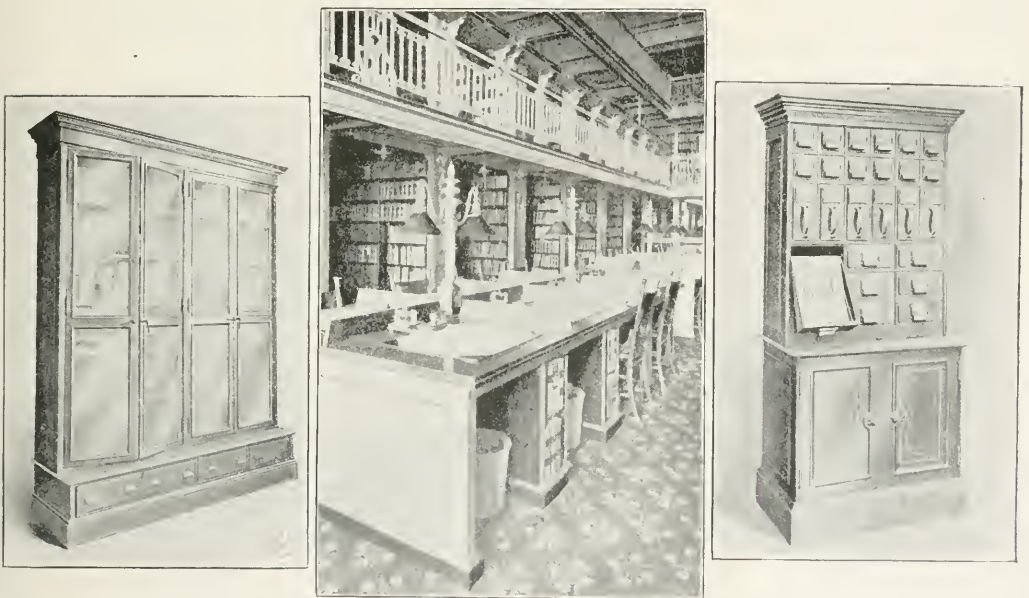


FIG. 71.—Iron furniture, Art Metal Construction Company, Jamestown, N. Y.

etc., iron work possesses unquestionable advantages; but the writer—though such an objection may be attributed to idiosyncrasy—cannot concede that iron cases are attractive. They seem distinctly repugnant to the senses of sight and feeling, and the most artful concealment of their metallic nature does not compensate for the warm tones, the more agreeable surface and the softer outlines of wood construction.

The iron case, however, must be reckoned with, and its use, especially in critical positions, will indubitably gain acceptance. The Art Metal Construction Company of Jamestown, N. Y., has perhaps done most to give iron a pleasing exterior, and their "Seven Points of Merit" are in many situations incontestible. They claim it is *sanitary, durable, convenient, agreeable, attractive, economical, incombustible.*

The admission, at any rate, is here freely made that for library equipment its claims are pervasive and commanding. That treacherous foe of the stored wisdom of the world — fire — has certainly in iron desks, racks, bookcases, chairs and tables a very formidable discouragement. Figure 71 shows the metallic library furnishings of the law library in the city hall, Philadelphia, and two examples of catalogue and book case.

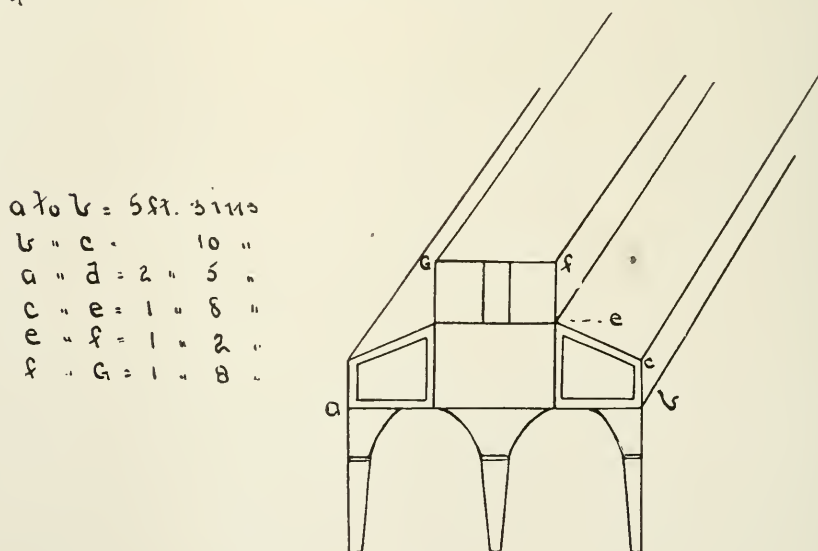
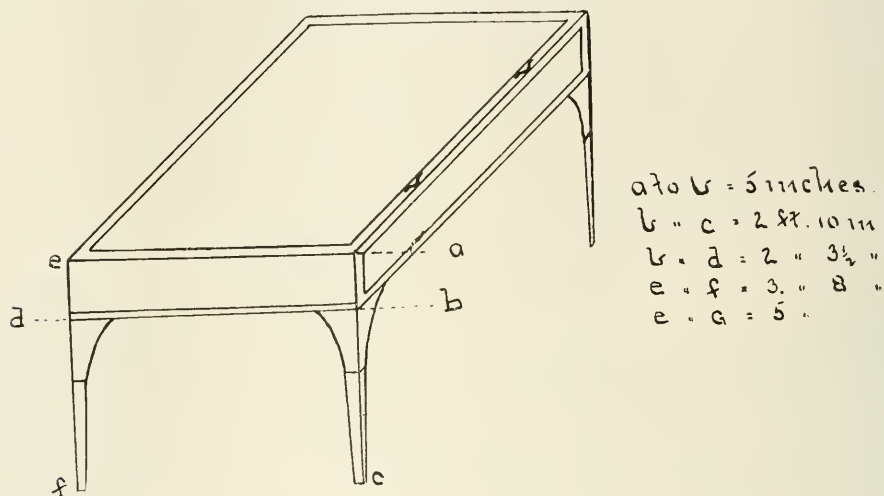


FIG. 72.

Examples of iron cases for museum purposes are given in Fig. 72 with dimensions. These cases were imported, and might be regarded as favorable instances of this kind of case, though in some respects their improvement is desirable.

A composite table case has been designed in the New York museum for the exhibition of lepidoptera. Figure 73 shows these cases with their contents on

the south side of the east tower hall. Generally, the height of the desk at front is three feet, and the length, made up of two sashes, eight feet; the width of the whole case, from the front of one sloping desk on one side, to the front of the opposite side, is 5 ft. 5 in. The steep A case between the desks is 2 ft. 2 in. high, and 18 ins. wide at the base. These cases are useful, but they scarcely meet the expectations of tasteful composition. Their design is mixed and inconclusive.

A table case of considerable interest, though overweighted with ornament, was designed for Prof. Henry A. Ward for the reception of his superb series of Meteorites—known as the Ward-Coonley collection. These cases are shown in Fig. 74. They are provided interiorly with stepped pyramids on which the

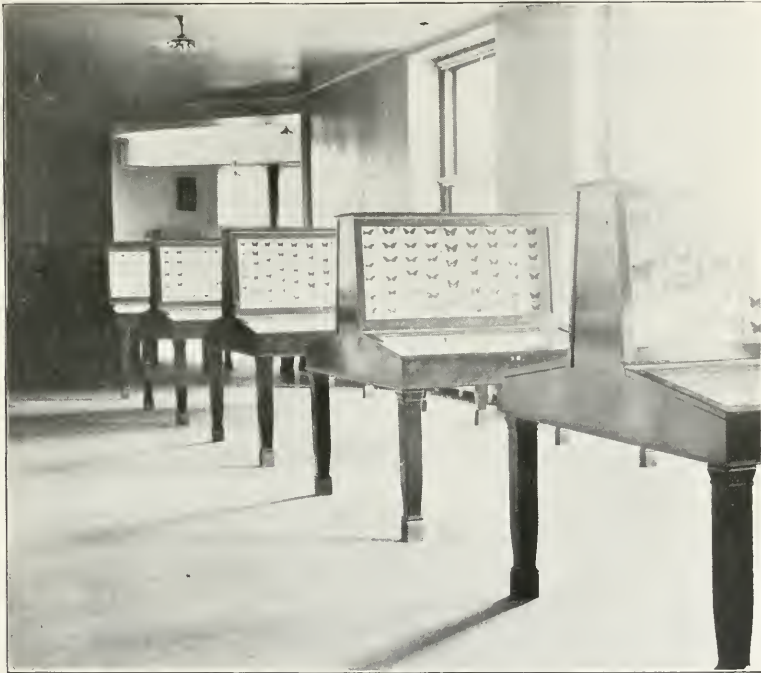


FIG. 73.—A and table case combined.

specimens are arranged in lines, raised on mahogany pedestals. The glass sashes are unlocked at the top and come out bodily from their positions, being lifted in and out, a disposition not favorable for convenient working, and attended with some danger, nor does it, in the instance illustrated, entirely preclude dust.

The question of dust is a very trying one to the museum curator, and all devices which can exclude this obnoxious, defacing and annoying substance are joyfully welcomed. Its complete exclusion seems to be rather a matter of *milieu* than of apparatus. Dustless positions for museums are a greater protection than elaborated safeguards against its penetration.

The prevalent method of protection in the table cases and to some extent in

the wall cases of the New York Museum is the use of green plush along the bearing surfaces of the cases and doors. A method in use in Toronto, in the mineral cabinet, is a tongue and groove (seen in Fig. 75), socketing into each other tightly, the frame of the sash being held rigidly in place by screw bolts in front and on the sides. The tongue is a triangular wooden cleat. Mr. Ferrier, whose collection is thus installed at Toronto, claims that it enjoys complete immunity from the invasion of dust in this way. The case is his own design.



FIG. 74.—Ward-Coonley collection cases.

A design, attributed to Prof. A. S. Bickmore, of a dustless case is shown in Fig. 76, where a close box is made, opening only at one end, allowing a false floor or body to be pulled out, a knee-leg supporting it when fully withdrawn. The serious defect in this case was the impossibility of placing such cases near together, and the very common vexation of finding the specimens disarranged when the false floor was pushed back, as, owing to irregularities of surface, etc., there were always jolts and hitches in its movement.

Recently the use of glass alone in the construction of cases has reached, at



least in show windows and shops, a quite popular recognition. The case is made of glass plates, riveted, bolted or clamped together, holding, in the best instances, a narrow strip of thin material, between their appressed edges. In such an arrangement there is of course no wooden sash, frame, etc., and the enthusiastic advocates of this system point out the clear view of all the contents of a case from any angle, and the disappearance of the heavy and clumsy equipments of wood, and the absolute dust-proofness of the cases, so made.

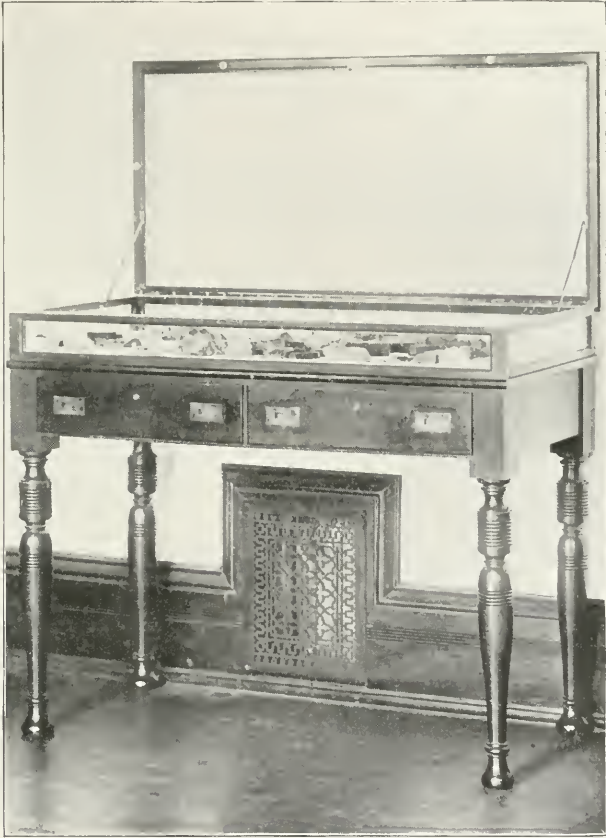


FIG. 75.—Table case, University of Toronto Museum.

I am indebted to F. X. Ganter, the proprietor of the Crystal All Glass show cases, for the very striking illustration of the contrast between the usual wooden-framed group case, and the glass case constructed by Mr. Ganter for the same purpose (Fig. 77). Mr. Ganter has been very successful in the adaptation of this system, and at his show rooms (Leadenhall and Stockholm streets, Baltimore) the serviceable and attractive examples of his manufacture can be readily inspected.

Certainly there seems an increasing possibility of the use of this system, in some measure, in museums. It might be attractively combined with the ordinary construction, and it has had and will have the salutary effect of diminishing

to the point of effective strength the width and thickness of wooden case-frames. Admission to desk cases and wall cases does not seem to be well provided for in it; but for groups, special mounts, permanent large exhibits, infrequently opened, it has a wide and hopeful applicability.

In this connection it is mentioned as forming a *dust-proof* cell. Examination was made of the front windows of a flower store in a dusty, wind swept avenue of New York city, and the assertion of the proprietor when asked as to the adequacy of this method of protection against dust, was that "not a particle of dust entered the store through the joints of the glass." These glass cases may very properly receive more and more attention from museums, and Mr. Ganter (397-399 Canal street, Baltimore) is desirous to introduce them into extensive scientific use.

Mr. Henry H. Hindshaw of the Albany State Museum has suggested a filter device by which free access of air to the case is allowed, robbed of its dust, thus

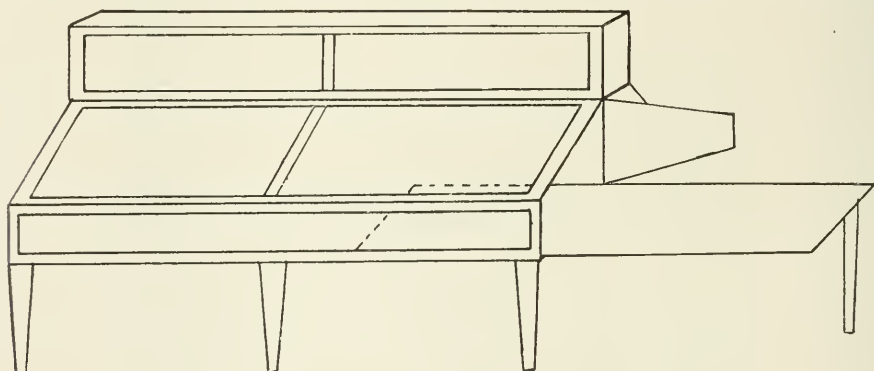


FIG. 76.

dissipating the effort of the air to enter the case from all sides owing to the circumambient pressure, which he avers is constantly present. The question of such a pressure seems apparently quite clear. In a series of experiments made in the New York museum, in which Mr. J. Maher assisted, the temperature, inside different cases, and under differing conditions, was found to be almost invariably higher than that of the air outside, producing obviously a pressure toward the case, though at moments this must almost disappear, and during much of the day cannot be appreciably great.

Temperature inside and outside of cases:

Feb. 25.		Feb. 26.		Feb. 27.	
Inside.	Outside.	Inside.	Outside.	Inside.	Outside.
70	76	63	68	68	72
72	76	63	64	69	72
72	74	65	66	70	73
73	71	65	66	70	74
		66	67	70	72
		66	68	72	74
		67	69	72	75
		68	70		
		68	70		

The times of taking the temperature were separated by an hour's interval; the first two tables were observed on two similar desk cases, respectively near and removed from a radiator, and the last table observed on a wall case. Mr. Hindshaw's contention receives confirmation.

The groove and tongue device is very thorough, but rubber for the latter is not permanently serviceable. It dries and loses coherence and after a time fails to fit the groove.



FIG. 77.

A thorough and comprehensive protection against dust is the introduction in the museum structure of the systems of ventilation involving filtered air. This of course means a greater initial expense, and it means, as a consequence, increased running cost. Such systems, rather sparingly introduced into buildings at first, are now more gratefully recognized as agreeable features. The air is propelled by fans, and comes in contact with wet surfaces of cloth or cotton batting, and is thus freed measurably of its dust burden. The ideal application of this system implies its use in summer as well as winter, which might seem to some an almost insuperable objection to its adoption.

Group cases are those cases especially adapted for individual exhibits, usually

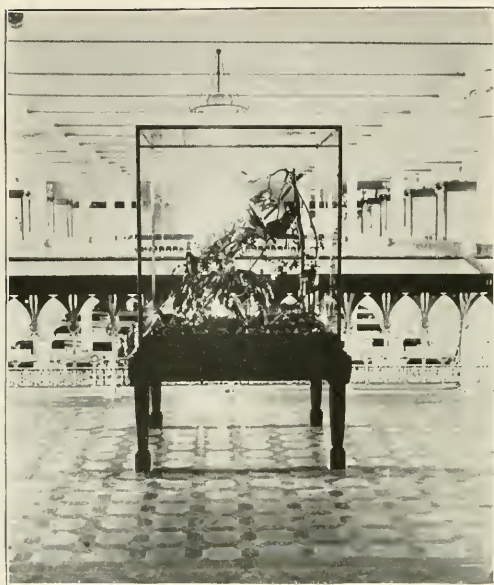


FIG. 78.—Group case with legs.

zoölogical, which may vary indefinitely from such mammoth boxes of glass as those which, in the New York museum, enclose the great groups of the bison and the moose, to anything small enough to accommodate the delicate architecture of a humming bird's nest.

Variation in cases of this type, in size, can be greatly extended, but it is evi-



FIG. 79.—Group case without legs.



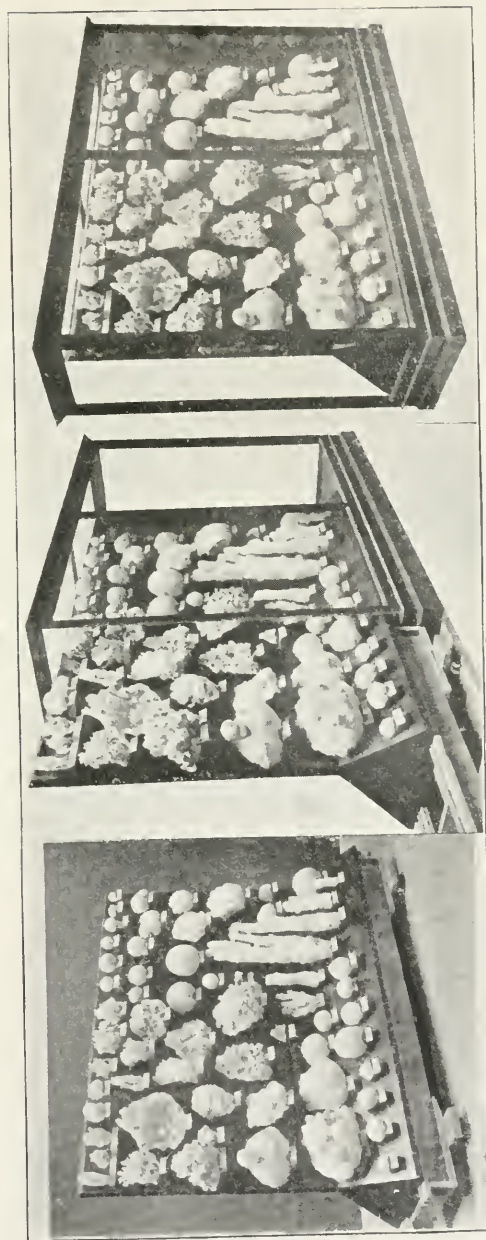


FIG. 80.—Original cases for corals, Field Columbian Museum.

dent that both variation and uniformity may be unwisely developed in a hall. Instances of the latter may be noticed in the Kensington Museum, London, and perhaps of the former in the National Museum, Washington. A very judicious result, in the use of various sized cases, is secured in the hall of vertebrate palæontology in the New York museum, and may be observed in Fig. 62. This will be further illustrated under Effect.

There are two contrasted phases in the construction of a Group Case, (1) the group case with legs like a table, (2) the group case with solid pedestal; Fig. 78 shows the former, Fig. 79 the latter. The objection to the former has been made that it suggests an absurdity, in that the ground space, or, if a water group, the pool, etc., of water, is in midair, while the pediment style brings both into



FIG. 81.—Nimrod Hall, Kensington Museum, London.

relation with the earth's surface. There is a suggestion of both hypo- and hyper-criticism in this. It is certainly not always convenient to build up a high pedestal, and pedestals of size are ugly objects, if high, while the supported group interferes less with the view of the hall floor, comports better with cleanliness, and practically obfuscates no one by its apparent aerial suspension.

In the two instances illustrated, the dimensions are for the former, on one side, 3 feet 8 inches, and on the other 4 feet, with glass sashes four feet high, the wooden moulding at the base  $7\frac{1}{2}$  inches high, with legs three to four inches square and 20 inches in height. The latter or pedimented case is 5 feet 11 inches to 6 feet on the sides with a slate base  $6\frac{1}{2}$  inches high, below a wooden moulding 8 inches high, with the surmounting glass box 6 feet 4 inches high.

The great moose group in the New York museum, composed and executed by John Rowley, is 29 feet long, 14 feet wide, 12 feet high, and is a very good example of case making on a house-building scale. It is encased on the long

side by four sashes, and on the short side by two, its top is covered by a wooden frame holding thirty-two ground glass lights.

Composite and original or specific cases are being constantly devised, and it would require a very greatly extended series of figures to represent the great diversity amongst them, or the special purposes subserved by them. The annual reports of museums are, fortunately, becoming rather valuable publications by reason of the profuse illustration, developed of recent years, in them. A chronicle of officers, patrons, gifts, and general statements of self-improvement is now usefully supplemented by figures of the interior and suggestive diagrams. The curator or director of a museum will find in these abundant examples of case work.

In the last annual report of the directors of the Field Columbian Museum there is shown what appears, to the writer, a faulty feature in cases, viz., the



FIG. 82.—Assyrian Hall, Kensington Museum, London.

elevation of their base by a few inches from contact with the floor. Cases should set squarely, completely, on the floor of the halls, and be protected by a slate base. Any *shallow* space beneath them furnishes a collecting spot for dirt, fragments, and paper, and only increases the difficulties of exacting cleanliness in the halls.

An exceptionally curious case, taken from the same report, is shown in Fig. 80, where the partition holding the exhibits slides outward. This again appears an awkward and unnecessary device, though perhaps the criticism would be modified upon inspection of the construction of the case. It is designed doubtless, as in the instance illustrated, to protect the delicate white corals from defacement by dust. It seems to possess this advantage undeniably. But unless easily manipulated it might offer opportunities for an excess of language quite irreconcilable with the appropriate moderation of scientific feeling.

Finally, without enlargement upon the subject in any greater detail, two illustrations are here introduced showing case dispositions in the Kensington Museum, London.

Figure 81 is the Nimrod hall in the Kensington Museum, and shows the table cases of rather an unattractive style and the wall cases with an open door.

Figure 82 is the Assyrian hall in the Kensington Museum, showing an upright glass floor case with a glass under case, which is injudicious, as all such floor enclosures are inaccessible and difficult to inspect at ease.

Under Accessories, figures of cases will be introduced helpful in forming plans, adjusting needs, and solving individual problems in installation.

It may finally be appropriately suggested that cases may be made in three ways, carelessly and cheaply, well and at a reasonable cost, over-elaborated and expensive. Of course the second procedure is the wisest. Rather than waste funds on ornamentation spend the money, saved by simplicity, on good glass. Secure neat proportions, use square legs under table cases, rather than round, turned legs. Insert table cases around galleries, supported by brackets; don't omit to put supporting arms under the lids of table cases, and get them of comfortable lengths, have the lids of cases project somewhat so as to be easily lifted, don't use handles, don't introduce a multiplicity of locks; one key should open every case in *one* hall, perhaps in many; Ienck's locks binding the two ends of a case door are probably the best. These bolt locks are now further supplemented by a flat key, which must first be used before the bolts move. The method is cemplicated, and does not work conveniently. If flat keys are used on table cases, have a lock at each end of the lid. *Keep out dust.* If table cases have a body of drawers underneath them, allow the top-case to project beyond the drawer cupboards. Have all drawers interchangeable by running them in on stepped cleets of whose height the depth of the drawer is a multiple. (See Accessories.)

Heavy iron wheels or rollers, hidden by a marginal skirt of wood or stone, should be in all cases, where feasible, placed under cases, where the latter are likely to be moved. I have seen the most unfortunate strains given to cases, and the most unlucky injuries inflicted on men by the hardship of having them *shoved* into new positions.

Movable bottoms for cases for bringing objects nearer to the eye are referred to under Accessories. Besides the cases we have enumerated, which embrace practically all the kinds really desirable in a museum, many small cases hanging on or fastened to the wall can be employed, in which single or unique groups of objects can be shown. Such cases can also be supported by brackets, and, if judiciously introduced, may form a most admirable feature in a hall. They should not be, however, interminably varied in size and treatment. Their uniformity, at least in each hall, contributes to their aggregate interest.

American Museum of Natural History.

L. P. GRATACAP.



## Methods in Plant Physiology.

### XVI.

(CONCLUDED.)

#### CHEMOTAXIS.

The response of free swimming organisms to chemicals is most conveniently studied by the use of mature antherozoids or motile bacteria. Fern prothalli bearing mature antheridia may usually be found after a little search in any greenhouse, or they may be grown by sowing spores in moist earth two months in advance of the time when required. Suitable prothalli should be placed upon slides and freed from earth by means of a dry brush and scalpel-blade; better results usually follow if they are allowed to dry for two or three minutes, then a few drops of rain water or filtered hydrant water may be added and the preparation covered with a cover-glass. If mature antherozoids are present they will be detected in a few minutes with the low powers of the microscope. Some prepared capillary tubes should now be ready to insert under the cover-glass.

The method of preparing the tubes is as follows: Heat a small glass tube in a flame and draw it out to a slender filament. Break up the thinnest part of the filament into pieces 8 to 10 mm. long and sketch them under a microscope with the aid of a camera lucida. By means of a stage micrometer measure the internal diameter of the tubes, selecting only those which are between .10 and .14 mm. and fusing up one end of each in a flame. They are then placed in a small dish of .05 per cent. neutral sodium malate (.05 per cent. malic acid neutralized with sodium hydroxid) and the air exhausted from them under the receiver of an air-pump. When the tubes are removed from the receiver some of the solution will enter them in place of the removed air.

Taking one of the tubes in a pair of small forceps, rinse it in water and insert it underneath the cover-glass, directing the open end of the tube to a place where antherozoids are seen swarming about. Note the entrance of the antherozoids into the tubes and whether any of them pass out again.

The same general method may be followed, using bacteria instead of antherozoids. A culture of bacteria may be obtained by boiling a pea for several minutes to kill it, and allowing it to putrefy in an open dish. Mount a drop of this culture liquid on a slide and insert, under the cover-glass, a capillary tube filled with a 1 per cent. solution of beef extract. Note the subsequent movements of the bacteria as they enter the area where the beef extract is diffusing out from the tubes. Study the reactions which ultimately bring them into the tubes.

#### AUTONOMOUS MOVEMENTS.

1. **Circumnutation of Stem Tips.**—The circumnutation of rigid stem tips is sometimes very small and a method must be employed which exaggerates the movement. The following method is taken from Darwin's *Power of Movement in Plants* and has been found to be very practical.

Place a potted *Coleus* in the bottom of a tall box which admits light only

from above and is covered by a large plate of glass. A glass filament, not thicker than a horse-hair, and from 3 to 5 cm. long, is fastened to the end of the stem by means of shellac dissolved in alcohol. If the solution be allowed to evaporate until it becomes so thick that it will set hard in two or three seconds, it will not injure the tissues. The end of the glass filament bears a very small bead of black sealing-wax. Below the bead is a small triangular piece of white paper, whose exact center is pierced by the filament. The bead and the paper triangle are viewed through the horizontal glass plate, and when the bead exactly covers the center of the triangle, a dot is made on the glass plate with a sharply pointed stick dipped in India ink. Make other dots at short intervals of time and join the successive dots with straight lines. In this manner a tracing of the movement of the stem tip will be obtained.

The circumnutation of twining plants can best be studied in-doors, where the wind does not interfere. On some twining plant select a shoot which has found no support, and whose apex has a large open hook. Thrust into the earth a wooden rod, and to its upper end tie the selected shoot, allowing the terminal 15 to 30 cm. of the shoot to project beyond the support. Place beneath the plant a sheet of white paper, and on it mark from time to time the position of the hook of the shoot, determining thus the direction and time of revolution.

Find other shoots that are twining about supports, and see whether the revolution of their tips has the same period as that of the free shoot.

Examine carefully the shoots that have twined and see whether the shoot shows torsion in climbing.

\* \* \* \* \*

In concluding this series of articles, the writer feels constrained to take this opportunity of expressing his thanks for the appreciative manner in which they have been received by a large body of teachers, and also to the editor of this JOURNAL for his painstaking care in presenting them to the reader in the best possible form. The aim, in giving these methods, has been toward practicability rather than originality. Although they come from a university laboratory, yet it is believed that many of them are well adapted to use in secondary schools. The writer will be very grateful to receive any criticisms or suggestions for making the methods more accurate and practical, and will be glad to give his attention to inquiries regarding them.

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## Laboratory Outlines for the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

XLIX. *Mosses, General Study.* Class, Musci. Order, Bryales.

(a) *The young gametophyte.*

When the nonsexual spore of a moss germinates it does not give rise directly to the scaly gametophore, but develops a green filamentous pro-embryo known as the protonema. The protonema can always be found in connection with the very young moss plants which are usually present in greenhouses. The protonema may also be found by examining some of the black earth from a place where mosses are growing. The ripe spores of any common species of moss may be sown on moist soil in a box. In a few days, if the box has been covered with a pane of glass, an abundance of green filaments will begin to appear.

1. Place a little earth with young moss plants into a watch glass and carefully wash off the soil by means of the medicine dropper and needle. Mount the plantlets and any minute masses of filaments present. Examine under low power. Find a good protonema and draw. Notice the branching, the shape of the cells, and the chloroplasts. The similar brown filaments present are rhizoids.

2. Draw a single cell, showing the wall, the cytoplasm, and the chloroplasts. Notice the oblique walls which may be seen in the older filaments. Where and how do the branches originate?

3. Find a protonema which has developed one or more solid green buds from which the mature sexual moss plants (gametophores) will develop. Draw.

4. With what kind of plants previously studied does the protonema compare? What then could you call the protonemal stage? How can this be used to explain the evolution of a moss as to habitat, form, and structure? Explain its evolution on this basis; remembering that the protonema is (1) a single cell, (2) a simple filament, (3) a branched filament; and that (4) it finally develops solid buds. These four stages represent the four successive steps in the evolution of the plant body in going from the lowest unicellular forms to the liverworts. Ontogeny is supposed to partly explain phylogeny. Learn the following law: The history of the development of the individual is an abbreviated history of the development of the race to which it belongs.

(b) *The young moss plant (gametophore).*

*Physcomitrium turbinatum* (nearly always abundant in greenhouses, by roadsides, and in old fields) or a species of *Mnium* will be suitable.

1. Mount in water and sketch the entire frond under low power, showing the stem, scales, and rhizoids.

2. Draw a single scale, carefully showing the costa and the margin. How does it differ from the scale of *Porella*? Under high power draw a cell showing the large chloroplasts and thick wall. As in the liverworts these scales are not homologous with true leaves.

3. Draw a branch of a rhizoid. How do these rhizoids differ from those of *Marchantia*? What relation is there between the rhizoids and protonema?

L. *Polytrichum commune* L. Common Hair-cap Moss.

Class, Musci. Order, Bryales. Family, Polytrichaceæ.

The common hair-cap is a widely distributed moss which grows on the ground in old fields and meadows, on hillsides and in woods. The plants are well preserved in a Mason fruit jar with 70 per cent. alcohol, and collections should be made at various times from winter until early summer when the capsules are mature. The plants are unisexual and the material for study should include mature male and female plants, female plants with the embryo sporophyte developed just far enough to rupture the calyptra, and female plants with mature or nearly mature sporophytes.

#### *Gametophyte.*

1. Draw the male and female plants (fronds) of the gametophyte generation, showing the rhizoids, scales, and tip. If the plants are dry or taken from alcohol, moisten in water. Note the rosette of scales at the tip of the male branch and also the scales at the tip of the female branch.

2. Take the tip of a mature male branch and dissect it with needles in a watch-glass, mount the detached parts and examine under low power. Notice the paraphyses and the white club-shaped antheridia. Do not mistake spatulate paraphyses for antheridia. Draw an antheridium under high power. Draw a spermatozoid from a ripe antheridium.

3. Study the living spermatozoids. These may be obtained if suitable male branches are collected after several days of dry weather. Take one of the branches and squeeze out the antheridia onto a slide. Mount in water and observe the motile spermatozoids.

4. If material is at hand, study and draw antheridia from a stained permanent mount.

5. Dissect the tip of a female plant, mount the detached parts from the center, and examine under low power. Draw an archegonium under high power, showing lid-cells, venter, and stalk. In good specimens the oosphere may be seen.

6. If convenient, study prepared slides containing archegonia. Draw, showing the stalk, venter, oosphere, neck, neck canal, and lid-cells.

7. Cut cross sections of the scaly stem of a large specimen (using elder pith and razor), mount in water and examine under high power. Draw, representing the epidermal layer, band of peripheral sclerenchyma, inner cortical layer of thinner-walled cells, and central stand.

#### *Sporophyte.*

8. Select a female plant with a young sporophyte, pull off the calyptra and then pull out the young sporophyte, being careful not to tear off its foot. Sketch the calyptra under the dissecting microscope. What does the calyptra represent? Sketch the young sporophyte under dissecting microscope, showing three



regions—foot, short stem, and tip. Remember that the mature sporophyte of *Marchantia* has three parts.

9. Draw a mature sporophyte of *Polytrichum*, showing the foot, the stalk or seta, the hypophysis, and the capsule or sporangium. What important advance has the sporophyte of *Polytrichum* and other mosses made over those of *Marchantia* and *Sphagnum*? Note that the sporophyte is a parasite during its entire life. Does it manufacture any food for itself?

10. Cut cross sections of the seta, mount, and draw under high power, showing the epidermal layer, the band of sclerenchyma, the layer of thin-walled parenchyma, and the central strand. The central strand of the sporophyte may be compared with the vascular bundle system of higher plants.

11. Ecological note. The hair-cap mosses are subject to great extremes of

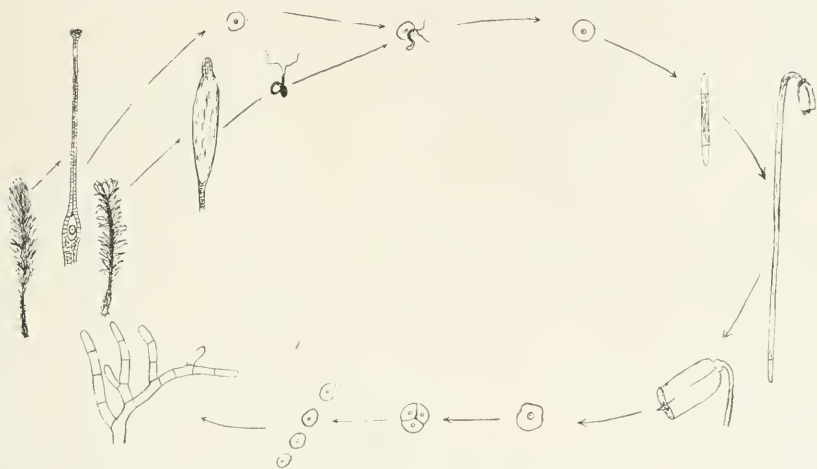


FIG. 9.—Diagram of life cycle of *Polytrichum*.

moisture and dryness. Let a gametophyte dry out and then place in water. What occurs? What adaptation has *Polytrichum* for checking the rapidity of evaporation?

12. Make a diagram in the notes showing the life cycle of *Polytrichum* (see Fig. 9).

LI. *Other Mosses*. (a) *Hypnum radiale* Beauv. (*Amblystegium varium* (Hedw.) Lindb.)

Order, Bryales. Family, Hypnaceæ.

This is a common moss on decaying logs, in moist, shady places, and on wet ground. Preserve in 70 per cent. alcohol.

*Sporophyte*.

1. Take a nearly mature sporophyte, lay it on a slide and examine without cover-glass under low power. Pick off the calyptra if still attached and the operculum, being careful so as not to injure the delicate teeth of the peristome. Draw the operculum and the calyptra.

2. Study the hygroscopic movements of the teeth of the peristome by gently breathing on the slide while making observations. Describe the movements. Of what use?

3. Study the true stomata on the hypophysis. Make a sketch of the capsule, showing the peristome and the hypophysis with stomata. The hypophysis may be compared with a leaf of the higher plants.

4. Cut open a capsule longitudinally and mount. Examine under high power and draw a stoma, showing the two guard cells and some of the surrounding cells. Draw several of the teeth of the peristome and some of the non-sexual spores. How many teeth are there? Are they in one or two circles?

5. Cut a cross section of a green capsule, mount and examine under low power. Sketch the section, noting the following structures: epidermis, hypodermal parenchyma, air space, spore sac, central columella.

(b) *Aulacomnium palustre* (L.) Schw.

Order, Bryales. Family, Bryaceæ.

This moss is common in boggy ground and may be found on charred logs and stumps or on the ground. Collect the material and grow in a moist chamber.

1. Under dissecting microscope make a sketch of a stem, modified in the upper part, the scales of which are easily detached. These scales act as brood buds, and when they fall to the ground are able to develop a protonema.

2. Mount some of the detached scales from near the tip and draw under low power.

(c) *Leptobryum pyriforme* (L.) Wils. Long-necked Bryum.

Family, Bryaceæ.

This interesting little moss may be found on moist, shaded cliffs and on rocks near water. It is very abundant in greenhouses, where the gametophyte may be obtained at any season. The rhizoids contain peculiar tuber-like brood buds of a dark-brown color. On the young sterile fronds these tuber-like bodies are often numerous, being produced on short rhizoids which come from the axils of the scales.

1. Mount and draw a mature brood-bud under high power, showing the structure. Describe.

2. Draw a rhizoid with an enlarged, light-colored end cell, the inception of the brood-bud.

#### A STUDY OF FORMS WHICH FORESHADOW SOME OF THE STRUCTURES DEVELOPED IN THE FOLLOWING SUB-KINGDOM.

LII. *Splachnum ampullaceum* L.

Class, Musci. Order, Bryales. Family, Splachnaceæ.

Although this odd looking moss is not very common, an attempt should be made to obtain fresh or alcoholic material of specimens containing mature or nearly mature sporophytes. The plant grows on decaying animal tissue or excreta and is said to occur in cranberry swamps from Ohio to New Jersey and northward.

1. Sketch the entire moss, showing the gametophyte and the sporophyte with the capsule and the very large, pyriform, fleshy hypophysis. Describe.
2. Sketch the capsule and hypophysis under low power, carefully representing the shape and surface details.
3. Examine the surface of the hypophysis under low and high power and note the stomata. Draw a small portion of the surface, showing stomata.
4. Note.—The large hypophysis covered with stomata and filled with loose tissue is well fitted for the work of photosynthesis and may be looked upon as foreshadowing the leaf structure found in the ferns and other higher plants.

### LIII. *Splachnum luteum* L. or *S. rubrum* L.

These remarkable mosses are rather uncommon, and very few will probably be able to collect specimens; nevertheless an effort should be made to obtain fresh or alcoholic material of plants with nearly mature sporophytes. They are reported mainly from the Rocky Mountain region.

1. Under low power make a careful drawing of the sporophyte, showing the foot, the seta, the large umbrella-like hypophysis, and the capsule (Fig. 10).

2. Draw part of the surface of the hypophysis under high power, showing the stomata. Are the stomata both on the upper and lower sides?

3. Compare the hypophyses of *Hypnum radiale*, *Polytrichum commune*, *Splachnum ampullaceum*, and *Splachnum luteum* and note the progressive development of the hypophysis as represented by these types. From this comparison it appears that the hypophysis may be regarded as a nascent, transpiratory and food-manufacturing organ.

### LIV. *Anthoceros laevis* L. or *A. punctatus* L.

Class, Anthocerotes. Order, Anthocerotales. Family, Anthocerotaceæ.

The horned liverworts are common on wet banks and sandstones ledges, especially around springs in shady places. The gametophyte is a small, lobed, more or less disc-shaped thallus from which the sporophytes extend upward like small vertical horns.

1. Under dissecting microscope, sketch a gametophyte containing nearly mature sporophytes. Note the thick tubular sheath around the base of the sporophyte.

2. Mount a small piece of the thallus and under high power draw a cell showing the single large chloroplast. Compare these cells with those of *Coleochaete*.

3. Look for endophytic colonies of a blue-green alga (*Nostoc*) in cavities on the under side of the thallus.

4. Separate a sporophyte, which is just mature at the tip, from the gametophyte, being careful to keep the foot in a perfect condition, and sketch under low



FIG. 10.  
Sporophyte  
of *Splachnum*  
*luteum*.

power. Represent the slender capsule, the bulbous foot with wart-like outgrowths, and the short stalk with a growing zone between the foot and capsule proper. Under high power note the stomata in the green tissue toward the base of the capsule. Draw.

5. Study a sporophyte in which the tip of the capsule has split open. Notice the columella.

6. Mount some of the spores and spore tetrads and draw under high power. Describe the spore tetrads.

7. If prepared slides are at hand, the details of the foot, the growing region, and the capsule should be worked out. Note especially the arrangement of the elaters, which have a tendency to separate the cavity of the capsule into transverse compartments.

8. Note.—The Anthoceroses come nearer to the lowest ferns than any other Bryophytes and it is probable that the Bryophyte ancestors of the lowest Pteridophytes were something like a horned liverwort with perhaps a chlorophyll-bearing tissue arranged somewhat like the hypophysis of a *Splachnum*. *Anthoceros* also points to the Pteridophytes in that it has the antheridia and archegonia embedded in the thallus.

In *Splachnum* and *Anthoceros* together appear five structures which foreshadow or anticipate important structures in the Pteridophytes. These are: (1) the bulbous foot and wart-like outgrowths of *Anthoceros*; (2) the central strand in the seta of *Splachnum* and other mosses; (3) the intermediate growing zone at the base of the *Anthoceros* capsule; (4) the large hypophysis of *Splachnum* with numerous stomata; and (5) the arrangement of the spores and elaters (sterile tissue) in the capsule of *Anthoceros*.

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## Industrial Microscopy.

### I.

#### INTRODUCTION.

Up to within the present generation the use of the microscope has been limited almost exclusively to investigations of a purely scientific character, and such instruments were but rarely found outside of the class-room or laboratory. In the high schools, until comparatively recent years, it has been regarded as little more than a scientific toy. At present, however, that attitude toward it is rapidly changing. The leading high schools now have courses requiring its use, while many of the schools consider one or more good compound microscopes a necessity for demonstration purposes. Its application in the arts and trades of the industrial world has also been marked. The merchantile world welcomes anything that will assist in determining the character of goods being handled. Of especial value has the microscope been in the examination of food products, drugs (herbs), fabrics, and paper making materials. Mention should also be made of its use in the great iron working establishments, which have specially equipped laboratories for the examination of steel and other forms of



iron. Under the impetus given to such applications by the work of such men as Moeller, Schimfer and others, many of our colleges and technical schools have courses involving practical applications of the instrument to certain branches of the trades and applied sciences.

Unfortunately, however, for the average American worker, the greater part of the literature upon the subject is in German or French. Although we cannot be expected to go exhaustively into the subject in such a series of papers as is here contemplated, yet letters of inquiry which the writer has received have led to the conclusion that the sources of such information are not widely known even among those who desire to know concerning the subject, to say nothing of that other class of individuals to whom the value of applied microscopy in the industries has never occurred. It is with a view to being of some service to these two classes of persons by collecting such material as may be available and putting into convenient form for the average beginner, that this series has been undertaken.

In the description of methods and technique we may treat the subjects from an elementary standpoint, but this fault will perhaps be pardoned in view of the fact that it is intended to be of service especially to the beginner in this line of work.

It may be well for the worker to bear constantly in mind that the use of the microscope is merely *applied* microscopy, that the basis of the whole subject lies in a knowledge of scientific facts, and the broader this knowledge the greater will be the ease with which results can be obtained. It really consists of gleanings in the scientific field gathered for a special purpose.

The greater portion of the substances which we have to deal with are directly or indirectly of vegetable origin. Hence it is quite necessary that the worker have a general idea of structural botany. Almost any of the text-books upon elementary botany give some attention to this branch of the study and will be of service.

Familiarity with the subject can only be gained by actual practice,—no amount of reading or examination of ever so accurate illustrations can take the place of personal examination of the substances themselves. Such can serve only as sign boards or danger signals, while the most intimate acquaintance of the features of the road must be gained by actually traveling over it.

U. S. Department of Agriculture.

BURTON J. HOWARD.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Juel, H. O. Ein Beitrag zur Entwicklungs-  
geschichte der Samenanlage von *Casuarina*.  
Flora. 92: 284-293, pl. 8, 1903.

Material of *Casuarina* sp. was collected  
in Algeria in January, 1901, and a col-  
lection of *C. quadrivalvis* was made at

Naples in March of the same year. The ovaries were carefully removed and fixed in the chromo-osmic-acetic mixture. The principal results are as follows: Each of the embryo-sac mother-cells, by two successive divisions, gives rise to four megaspores. The cells of the archesporium are distinguished by their larger nuclei and denser contents. The first division in the megaspore mother-cell is marked by the usual synapsis stage and a reduction in the number of chromosomes. The number of chromosomes at this division was not determined definitely, but was not less than 8 nor more than 12, while the number in sporophytic cells was about twice as large. Bodies of kinoplasmic aspect appear at or beyond the poles of the spindle during the mitoses which give rise to the four megaspores, but these bodies are not to be regarded as centrospheres. They resemble the dense areas which have been described in various Gymnosperms.

The later stages were not studied, but the writer remarks that in regard to the development of the embryo-sac, the entrance of the pollen tube and the formation of the embryo, he can only confirm the account of Treub. c. j. c.

Grout, A. J. Mosses with a Hand Lens and  
Microscope, a non-technical hand-book of  
the more common mosses of the northeast-  
ern United States. Part I, pp. 1-86, with  
10 plates and 35 text figures. Published by  
the author, 360 Lenox Road, Brooklyn, New  
York City, 1903. Price, \$1.

The success of Prof. Grout's little book,  
*Mosses with a Hand Lens*, has led him  
to publish the present work. It makes  
no pretensions to being a complete  
manual, but, rather, is intended to  
attract and help students who would

otherwise never begin the study of mosses. While the specialist who studies species critically needs something more extended and critical, even he may find the book convenient for the ready determination of miscellaneous collections. About one-half of Part I is occupied by the following subjects: The collection and preservation of mosses, how to mount mosses, methods of manipulation, life history and structure of the moss plant, and an illustrated glossary of bryological terms.

Many of the elegant plates are taken from the "Bryologia Europea," some from "Sullivant's Icones," while a goodly number are original. The descriptions of families are rather full and are accompanied by numerous illustrations of the characteristic features. The classification follows quite closely that in Dixon and Jameson's "Handbook of British Mosses."

Part I contains descriptions of Sphagnaceæ, Andreaeæ, Georgiaceæ, Polytrichaceæ, Buxbaumiaceæ, Fissidentaceæ and Dicranaceæ. The book should receive a hearty welcome from teachers and students. c. j. c.

**Lignier, O.** Le fruit du *Williamsonia gigas* Carr. et les Bennettitales, documents nouveaux et notes critique. Mémoires de la Société Linnéenne de Normandie, **21**: 19-56, 1903.

The reinvestigation of this remarkable fossil was suggested by Wieland's researches upon *Cycadoidea*, and there seems to be considerable resemblance

between the two forms. According to the present account, the structures in *Williamsonia gigas* which have been described as "male flowers" are really the axes of ovulate strobili from which the layer of ovules has become detached after maturity. The staminate structures were probably comparable to those described by Wieland for *Cycadoidea ingens*. The fruit of the Bennettitales should be considered not as a flower but as an inflorescence.

A diagram showing the relationships of great groups is submitted. From the Protopteridæ, the ancestors of the Filicales, is derived a stock which becomes differentiated into two main lines, the Salisburiales and Cordaitales. At an early period the Cycadales were derived from the Salisburiales and, later, the Coniferales came from the same stock. From the Cordaitales at an early period came the Bennettitales and, later, the Gnetales and Angiosperms. More must be known of the life history of fossil forms lying between Pteridophytes and Gymnosperms, and also of fossils in these two groups before a satisfactory diagram of relationships can be constructed.

C. J. C.

**Rosenberg, O.** Das Verhalten der Chromosomen in einer hybriden Pflanze. Ber. d. deutsch. bot. Gesell. **21**: 110-119, pl. 7, 1903.

So little cytological work has been done upon plant hybrids that every contribution excites more than ordinary

interest. It is well known that the various species of a given genus generally have the same number of chromosomes. Prof. Rosenberg has been fortunate in finding a hybrid between parents which differ from each other both in the number and size of their chromosomes. The hybrid is a cross between *Drosera rotundifolia* and *D. longifolia*. *D. rotundifolia* has 20 chromosomes in the sporophyte, this number appearing in the stem, leaf and root. The chromosomes are short and easy to count. In the pollen mother-cell the number is always 10. In *D. longifolia* the vegetative tissues show 40 chromosomes and the pollen mother-cells 20, the chromosomes being somewhat smaller than in *D. rotundifolia*. The chromosomes were not counted in the megaspore mother-cell of either species.

The hybrid is easily recognized by external characters, but is also characterized by its chromosomes. The mitoses are not different from those of the parents except in the number of chromosomes and consequent variation in the shape of the spindle. In the sporophyte 30 chromosomes—the sum of the gametophyte numbers of the two parents—was counted in the root, stem and leaf. In a few cases 40 chromosomes appeared in tapetal cells. In the pollen mother-cells 15 chromosomes is the dominant number, but 20 often occur and occasionally mother-cells with 10 are found. All three numbers have been found in the same anther. The megaspore mother-cells were not investigated.

The fact that three kinds of pollen grains are formed has its bearing upon Mendelism. The relative numbers of chromosomes in the parents and hybrid support the theory that the chromosome is a permanent organ of the cell.

C. J. C.

# CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE MOODY.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole Moody, 125 Belvedere Street, San Francisco, Cal.

**Nemiloff, Anton.** Zur Frage der amitotischen Kernteilung bei Wirbeltieren. Anat. Anz. 23: 353-367, 1903.

The author limited his observations to vertebrate material and used for direct division, as most favorable objects, the

giant cells of the epithelium of the bladder and the lymphoid layer of the liver amphibia. In the first the object is very favorable, but shows no centrosome nor attraction sphere. The second object is unfavorable owing to the comparatively small size of the lymphoid cells; yet the relations of the central body to nuclear division is very clear.

The mouse was principally used for the giant cells of the bladder since the giant cells are quite large, though thin. A chloroformed mouse was quickly opened, the bladder cut lengthwise and fixed to cork with thin needles, with its inner surface out. A cover-glass, cleaned with absolute alcohol and ether, is quickly pressed on to the mucous surface. The epithelial cells stick to the sides as in paraffin sections. The cover is put into fixatives after being fastened to a piece of cork to keep it afloat. After fixation the cover can be treated in the customary way. The stains used were Heidenhain's hematoxylin, saffranin with light green or picric acid, toluidin blue with erythrosin. In cells so prepared many division stages are found. In resting one or more nucleated cells the nuclei are round or almost round, the nucleolus is in the middle, surrounded by a light zone, the significance of which is puzzling. With the approach of division the nucleus and nucleolus elongate; the latter constricts in the middle and finally divides into two spherical parts. The light zone divides after the nucleolar process has ended. Nuclear division begins sometimes simultaneously with nucleolar division and sometimes after it is completed. It is a process of constriction similar to that of the nucleolus. The two daughter nuclei are spherical and somewhat flattened. Between the opposed surfaces of the nuclei now appears a characteristic lining or striation of doubtful significance. This internuclear substance stains with iron hematoxylin, but by prolonged bleaching in alum becomes almost invisible, if slightly bleached it looks granular. It is not possible that these appearances are artifacts, as they appear in some stages and are absent from others on the same slide. The author sees a suggestion in this of the processes of mitotic division. Preparations fixed in Lenhossek's mixture and carefully stained with saffranin or iron hematoxylin show chromatin in resting and dividing nuclei. Certain figures were found resembling Carnoy's chromatic canals; these lengthening out on the division of the nucleus were replaced by the internuclear fibrous substance already spoken of. The lymphoid cells of the liver of amphibians show three types of division, karyokinetic of the typical kind, indirect, also the usual form, and a third of the following nature: a funnel formed depression appears on one side. At the base of the depression lies a granule that stains deeply, sometimes it is surrounded by radiating lines, showing it to be a centrosome. These radiations are seen to connect with the nuclear substance. This depression deepens until finally the nucleus is ring-shaped with the centrosome in the middle. Gradually the ring thins at two opposite points and two nuclei are formed, or there may be several. This forms an intermediate type.

A. C. M.



## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

**Monti, R.** Le funzioni di secrezione di assortimenti intestinale studiate negli iber-nanti. Mem. nat. Istituto Lombardo. 1903, pp. 1-34, 2 pls.

For the study of the intestinal epithe-lium of hibernating mammals the author found that many of the methods of

modern technique were poorly adapted. For example, Carnoy's fluid failed to give good results. Mingazzini's sublimate-alcohol-acetic, Zenker's, Hermann's, and Flemming's mixtures are recommended. The most delicate fixation, how-ever, was obtained by Perenyi's fluid, to which a few drops of acetic acid had been added. Certain structural features were best demonstrated by fixing in Moeller's mixture, 40 parts of 3 per cent. bichromate of potash and 10 parts of 40 per cent. formalin (not formaldehyde). The tissues were left for 24 hours in this mixture and then transferred to 3 per cent. bichromate of potash for 3 to 4 days, washed for 3 hours in running water and transferred to alcohol. Some tissues fixed in osmic-bichromate for 3 to 4 days, washed for a "long time" in running water until no more color was given off and then embedded, gave in very thin sections the finest differentiation, even with ordinary stains.

For staining, Bizzozzero's method and thionin-erythrosin were used for demon-stration of muciparous cells. The methods of Biondi, of Galeotti, and the tria-cid of Ehrlich were used for the glands of Brunner and Lieberkuhn, and Heiden-hain's iron hæmatoxylin with counter-stain of acid fuchsin, Bordeaux red, or rubin were used for most of the preparations.

C. A. K.

**Thilenius, G.** Ergebnisse einer Reise durch Oceanien. Zoöl. Jahrb. Abth. f. Syst. 17: 425-444, 1902.

The author writes from several years' experience in collecting zoölogical material by the most approved German

methods for subsequent investigation by modern laboratory methods. The paper is replete with suggestions as to general methods employed in the field, as to outfit in matters of implements, instruments, guns, photographic supplies, chemicals, and preservation and packing of the collections. A few items only can be noticed here. For fixing fluids the author recommends, for simplicity and rapidity, hot water, formol, alcohol, sublimate and picric acid. Chromic acid and its salts are to be avoided on account of the long after treatment, the ease of maceration and of breakage in handling. Picric acid is especially appli-cable since prolonged exposure to it is not deleterious. Reptile embryos kept for 2 years in the saturated aqueous solution were found to be in good condi-tion. The author has high praises for formol, used properly, as a general pres-ervative. For vertebrates it should be injected through the aorta or carotid. Mere opening of the body cavity rarely suffices, though if this is supplemented by injection of the digestive tract, it will preserve smaller animals well. Fle-shy fishes and reptiles should also have the musculature injected. The author highly recommends formol for use with small mammals and birds in the tropics where the exigencies occasioned by high temperature and lack of time for proper care of material results frequently in great loss. Such animals are eviscerated with care, the cornea, mouth, and shot-holes carefully wet with strong formol, and a wad of cotton saturated with the same is placed in the body cavity. Such animals carefully packed make good preparations after a full year's preservation. Weaker solutions of formol (10 per cent.) may be similarly used for temporary preservation.

C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory,  
University of Michigan, Ann Arbor, Mich.

von Linden, M. Grafin. Morphologische und physiologisch-chemische Untersuchungen über die Pigmente der Lepidopteren. Arch. f. d. ges. Physiol. 98: 1-89, 1903.

In this first, very detailed and thorough contribution to a subject of great interest, the author deals with the yellow and red pigments of the *Vanessa* (io and urtica). She finds that the red or reddish yellow pigment of the scales of these insects is found in different modifications of color in the bodies of the pupæ as well as in the tissues of the caterpillars. The color tone of the pigment depends on its degree of oxidation, and by changing the amount of oxygen a series of color changes may be produced, as is the case with bile pigments. The reduced pigment is carmine red in color, while oxidation changes it to a yellow-green-gray color. In the tissues of the caterpillar the pigment is found in its oxidized form. The carmine red form first arises after the pupa case has hardened, practically completely shutting off the epidermis from the oxygen of the air. The carmine red pigment changes within the scales themselves into its yellowish red modification. The chemical rays of the sunlight act as oxidizing agents, changing yellowish brown solutions to greenish grey. Heat darkens the color, bringing out a red-brown tone. The *Vanessa* pigment is capable of crystallization, the crystals being clinorhombic plates. The crystals are very much like those of hæmatoidin and bilirubin. The pigment has a characteristic absorption spectrum, much like that of urobilin. It is a proteid body, reacting clearly to the Millon and xanthoproteic tests. It is easily shown to be a combination of a proteid body with a pigment, such as we have in hæmoglobin for example. The proteid body is an albumose, apparently allied to the globulins. The colored component of the pigment is an acid, resembling bilirubin in its reactions with solvents, its crystal form, color and optical relations. Its spectrum, on the other hand, is more like that of urobilin or hydrobilirubin. The red *Vanessa* pigment contains iron and free sugar. On this account the author thinks that it has some function as a reserve substance. Its principal function is in respiration. The pigment is formed in the intestine from plant pigments (principally chlorophyll) taken in with the food.

The paper, though full of detail, is extremely interesting, and stands as an example of the sort of investigation which must be prosecuted before we can hope to get far with the problem of animal coloration.

R. P.

Prochownik, S. Ueber Widerstands- und Lebensfähigkeit epithelialer Zellen. Zeitschr. f. Allgem. Physiol. 3: 33-56, 1903.

The author has made a study of the ability of certain epithelial tissues of mammals to withstand various harmful environmental exposures, and at the same remain alive and capable of regeneration. His results are very remarkable. The method of experimentation used

was, in outline, as follows: a small portion of some gland, usually one of the salivary glands, was excised under as nearly as possible aseptic conditions. The piece of tissue was then kept out of the body and exposed to the environmental condition it was desired to test (e. g., heat, cold, chemical agents, etc.). Then it was transplanted into the kidney or under the peritoneum of the same animal, or some other animal of the same species. After some days the area of transplantation was removed and sectioned, and search was made for evidence of regeneration in the transplanted tissue. The occurrence of regeneration was, of course, proof that the tissue had successfully withstood the environmental difficulties. The animals used were rabbits. Examples of the results obtained follow. A piece of salivary gland kept dry in a refrigerator for 43 hours showed regeneration after transplantation. A similar piece kept moist in a refrigerator for  $54\frac{1}{2}$  hours showed regeneration. Experiments with mammary gland tissue and with pieces of the pancreas failed entirely. A piece of testis showed regeneration after a stay of 48 hours in a refrigerator. Experiments in which the pieces of tissue were immersed in chloroform or in formalin (10 per cent.!) failed to give positive results. Pieces of salivary gland and of testis after exposure for from 15 to 20 minutes to a temperature of  $60^{\circ}\text{C}$ ., showed marked regenerating capacity. A piece of salivary gland regenerated after 45 minutes exposure to a temperature of  $-21^{\circ}\text{C}$ ., and pieces of testis showed similar resisting power to such extreme cold. Pieces of the same tissue solidly frozen on the stage of a freezing microtome regenerated. These results bring out very forcibly the fact that maximum and minimum temperatures within which life is possible are very different for the cell and for the organism as a whole.

R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt, Harvard University Medical School, Boston, Mass.

Wechsberg, F. Beitrag zur Lehre von der primären Einwirkung des Tuberkelbacillus. Ziegler's Beiträge, 29: 203-232, 1901.

Baumgarten has maintained that the production of miliary tubercles and diffuse tuberculous tissue is the result

of a direct irritation by the tubercle bacillus of the fixed cells of the part. This irritation causes the cells to proliferate.

According to Weigert's well known theory such an explanation would not be tenable. The cause of the proliferation of the cells should be sought rather in an injury to the tissue which removes the inhibitory force that normally holds the power of growth of the cells in check. Neither Baumgarten nor the other writers have concerned themselves with the existence of such a primary injury.

Wechsberg studied this problem in Weigert's laboratory. Suspensions of tubercle bacilli were injected into the ear veins of rabbits. The animals were killed at different periods, ranging from six hours to twelve days, and the lungs hardened and sectioned.

At the end of six hours the blood-vessels showed in places a loss of their endothelium as well as a marked destruction of their elastic fibers. Groups of bacilli lay in the lumina of the vessels, surrounded by polynuclear leucocytes. After twenty-four hours epithelioid cells were found. The destruction of elastic tissue was the more extensive the longer the duration of the infection.

Wechsberg concludes that the tubercle bacillus destroys the fixed cells and the intracellular tissue by its toxic action. It first injures the new formed cells so that they are unable to produce connective tissue and blood-vessels. The formation of giant cells indicates that the protoplasm of the proliferated cells is only injured in part. Finally the new formed cells become entirely destroyed and the stage of caseation is reached.

J. H. P.

**Herxheimer, G.** Ueber die Wirkungsweise des Tuberkelbacillus bei experimenteller Lungentuberkulose. *Ziegler's Beiträge*, **33**: 363-407, 1903.

Recently an elaborate monograph on the mode of action of the tubercle bacillus has been written by one of Weigert's assistants. It is in a sense a continuation of the study begun by Wechsberg.

Tubercle bacilli suspended in fluid were injected into the trachea of rabbits and guinea pigs. The animals were killed at different intervals, from one-half hour to seven weeks after the injection. The staining method which gave the best results was a combination of Weigert's elastic tissue stain and anilin-water-methyl-violet. The tubercle bacilli were colored blue and stood out sharply upon the red background.

1. Stain the sections in lithium carmine several minutes.
2. Differentiate in 1 per cent. hydrochloric acid alcohol several hours.
3. Stain in Weigert's elastic stain one hour.
4. Wash rapidly in acid alcohol.
5. Differentiate quickly in 96 per cent. alcohol.
6. Stain in anilin methyl violet several hours in the cold.
7. Differentiate in acid alcohol and in 96 per cent. alcohol.
8. Dehydrate in absolute alcohol.
9. Clear in xylol.
10. Mount in balsam.

In order to remove the violet color from the celloidin the sections, after treating with absolute alcohol, were dipped in an alcohol-ether mixture which dissolves away the celloidin.

The bacilli, as soon as they reached the lung, were taken up by the epithelial cells lying free in the alveoli, and by cells still attached to the alveolar wall. Desquamation of the epithelial cells soon followed the ingestion of the bacilli. At the same time the bacilli exert a destructive influence upon the elastic fibres. A result of the injurious action upon the cells and the intercellular substance is a proliferation of the fixed elements; a growth of epithelium occurs as well as of endothelium and connective tissue cells.

J. H. P.



## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn, Wesleyan University, Middletown, Conn.

**Lafar, Dr. Franz.** Technical Mycology. Vol. II, pt. I, pp. 189. Figures 67. Published by J. B. Lippincott & Co., Philadelphia.

All acquainted with the first volume of Lafar's Mycology will welcome this first part of volume II. Although the part

now issued does not complete the second volume, the English publishers have had so many calls for the companion volume to the one already published that they have issued the first part separately instead of waiting for the completion of the work by the author. The rest will be translated as soon as possible. The new volume just issued deals with Eumycetic fermentation. Three chapters are devoted to the general morphology of the moulds and their allies, their chemical composition and the enzymes produced by them. The rest of the work is confined to the study of the Mucors and yeasts. As indicated in the title the subjects especially dwelt upon are those which concern the practical relations of Mucors and yeasts to industrial operations. Nevertheless, enough of general morphology and chemistry is given to make the work useful to the scientist as well as to the technical student of fermentative industries. The use of yeasts in fermentation is of course well known, but most readers will be somewhat surprised to learn that the Mucor family is also of considerable practical use in various fermentative industries. Lafar has an exceptionally clear style of writing and shows an ability of selecting for description and explanation exactly the questions that are likely to puzzle the student. The work is a very valuable one and should find its way to the bookshelves of all bacteriologists. H. W. C.

**Holliger.** Bakteriologische Untersuchungen über Mehlteiggärung. Cent. f. Bact. u. Par. II, 9: 305, 1902.

This author makes a very interesting series of studies upon the fermentation of bread dough for bread raising, by

the use of sour dough (leaven) and ordinary yeast.

He finds that in all cases both bacteria and yeasts are concerned. The bacteria are in such cases mostly lactic bacteria which produce no gas, and do not contribute to the raising. They do have a decided utility, however, in preventing the growth of various putrefying bacteria which are sure to be present and produce trouble if not checked by the development of acid. The author says that in a similar way the growth of the yeast prevents the development of molds which would otherwise produce trouble. The chief difference between the bread raising by the use of commercial yeast and the use of leaven is in the quickness and certainty of the results. The yeast contains a larger number of yeast organisms and acts, therefore, more quickly and more surely. As a result, the use of such yeast is rapidly supplanting the older more simple method of raising bread by leaven. But even yet in rural localities the older method is in most common use. Holliger studies also the spontaneous

fermentation of dough and finds this a different phenomenon. The fermentation is in this case not produced by yeasts but by two species of bacteria both of which produce gas.

H. W. C.

**Klopstock.** Beitrag zur Differenzierung von Typhus, Coli und Ruhrbacillen. Ber. klin. Woch. 803, 1903.

This work tests and expands the method of differentiating typhoid bacilli first advanced by Barsickow. This method uses two media, each containing nutrose, salt, and water and either milk sugar or glucose. Klopstock combines the two and obtains a medium which he regards as satisfactory in separating typhoid, colon, and dysentery bacilli. By putting both milk sugar and glucose into a medium composed of nutrose and salt, and inoculating with these different bacilli in fermentation tubes he determines the following diagnostic characters. The dysentery bacillus produces simply an acid reaction. The typhoid bacillus produces an acid reaction and a curdling, while the colon bacillus produces acid, curdles, and forms gas.

H. W. C.

**Behrend.** Nachprüfung zwei neuer Methoden der Geisselfärbung bei bakterien. Hyg. Hund. 691, 1903.

The author compares the methods of flagella staining devised by Rossi and Peppler. The former uses as a mordant concentrated tannin solution and as a stain Ziehl's solution diluted. This stain gave unsatisfactory results, as Behrend believes, because of the weak action of the mordant. The method of Peppler uses a mordant of saturated tannin, to which is added 2.5 per cent. HCl, and as a stain carbolgentian-violet. The use of this mordant for three minutes, followed by the stain for two minutes, gives excellent results. He finds the most favorable objects for staining are eight to thirteen hour cultures.

H. W. C.

**Cambier.** Note sur une nouvelle methode de recherche du bacille d' Erberth. Rev. d'Hyg. 64, 1902.

The author adds another to the numerous methods of separating the typhoid bacillus from *B. coli* and other similar organisms. It depends upon the difference in rapidity with which the organisms will grow through a porcelain filter. He prepares a solution of

1000 c. c. 3 per cent. solution of peptone

80-100 c. c. 1 per cent. NaOH

88-120 c. c. saturated salt solution.

The suspected material, e. g., feces, in solution is placed inside the candle of a Chamberland filter F., and the lower part of the candle placed in the solution above described. The whole apparatus is incubated at 37°, and, if typhoid bacilli are present, the bouillion becomes cloudy in a few hours and is found to contain nearly a pure culture of typhoid bacillus.

For testing water he first filters many liters through a similar filter, and then places the filter in the solution in the same manner.

H. W. C.

## GENERAL LABORATORY TECHNIQUE.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory, University of Michigan, Ann Arbor, Mich.

### A Mechanical Rocker for Developing Negatives.

In *American Medicine* for August 22, 1903, p. 303, Dr. G. E. Pfahler describes a very neat device which he has

invented for rocking the developing pan during the development of photographic negatives. The apparatus was devised especially for use in Röntgen-ray work, but will be found equally useful in all sorts of laboratory photographic work. The apparatus consists of the motor of an electric fan, from which the fan has been detached. This is done by loosening a single screw, and in place of the fan is attached a pulley 1 inch in diameter. The belt then runs over a wheel

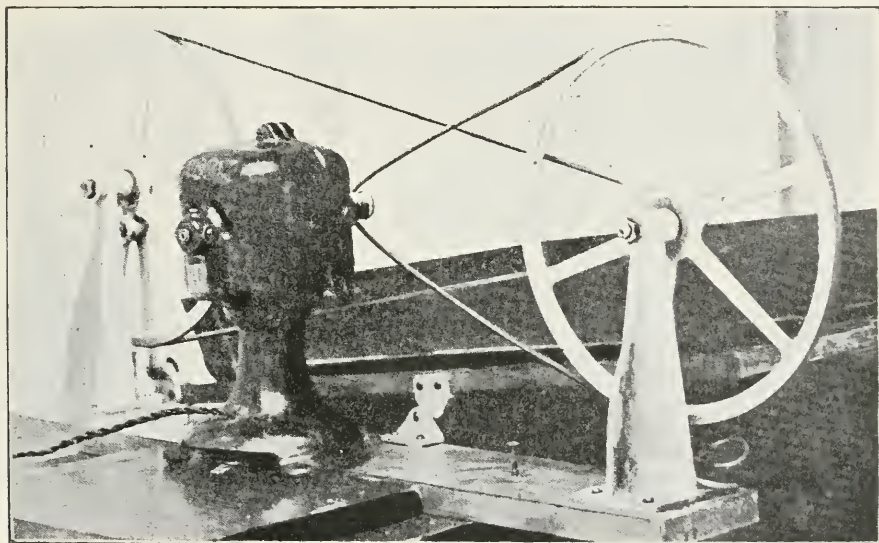


FIG. 1.

12 inches in diameter to which a pulley 1 inch in diameter is attached. This turns a wheel 12 inches in diameter having an arm attached  $1\frac{1}{2}$  inch from the center, which gives the rocking base a sway of 3 inches. The pulley on the motor revolves 1000 to 3000 times per minute. This rocks the pan from 10 to 20 times per minute, according to the regulation of the amount of the current. The pulley on the motor can be replaced by the fan in less than half a minute, which can then be used to fan the plates dry. Details of the apparatus are shown on the illustration, Fig. 1.

R. P.

### Schultze's Method of Preparing Vertebrate Embryos to Show Ossification.

Preparations of embryos made by Schultze's method, in which the ossified portions of bones appear as white

opaque objects, while all other tissues are transparent, are among the most striking and useful of embryological preparations. The method of making them seems not to be generally known, and as the only place where it is published (so far as known to the reviewer) is in Schultze's "*Grundriss der Entwicklungsgeschichte des Menschen und der Säugethiere*" (Leipzig, 1896). it seems desirable

to republish the method in order that the readers of this JOURNAL may have the benefit of it. Schultze himself says regarding it that "it is the most elegant and best method for studying the macroscopic phenomena in the formation of bone, and frequently makes superfluous the tedious method of serial sections."

Embryos should be hardened in alcohol for at least 8 days. (Embryos fixed and hardened in *acid* solutions cannot be used in this method.) From alcohol specimens are transferred directly to an aqueous solution of KOH of a concentration of from 3 to 5 per cent. In case the embryos are large the brain should be taken out through the great fontanelles with fine forceps and needles, and the thoracic and abdominal viscera should be removed through a median longitudinal incision along the ventral body wall. After a period of time which varies in length according to the size of the specimens, the duration of the alcohol hardening and the concentration of the KOH, the embryos become transparent. They may be then permanently preserved in the following solution:

Water	-	-	-	-	-	100 parts
Glycerine	-	-	-	-	-	30 parts
Formal (35 per cent.)	-	-	-	-	-	2 parts

In these preparations every point where ossification has taken place appears ivory white in color and opaque, while all other portions are transparent or translucent.

R. P.

### Question: "Why do Some Crystals Polarize, and Some Not, e. g., Common Salt, etc.?"

A ray of white light is a complex bundle of waves of varying forms and rapidity of motion.

Polarization by a crystal or other substance is merely the revelation to the eye, by colors, through the use of a pair of nicol prisms as a test agency, of disturbance so affected in the ray of white light, during its transmission through the particular substance. When such a ray is passed through a transparent substance, its path may be broken (simply repeated), but the ray itself may pass otherwise entirely undisturbed through certain substances. In such cases, the substance among whose molecules the ray passes unaffected is termed "simply repeating"; merely its path broken and no colors of polarization produced (e. g., air, water, glass, common salt, garnet, and all substances crystallizing in the Isometric system, under ordinary conditions of pressure).

Nor is the ray even affected by "doubly refracting substances" (e. g., all other crystals but those of the Isometric system), when passed through them in certain definite crystallographic directions, called the "optic axes." In neither of these cases do colors of polarization appear, when the ray is tested between nicols whose planes are crossed, i. e., producing a dark field.

On the other other hand, when the ray passes through "double refracting" crystals in any other directions than those of the "optic axes," or through any transparent substances whatever, when these happen to be under strain, or through transparent substances of finely laminated or finely radial structure (e. g., sections of pearl, opal, chalcedony, ruled gratings on glass, etc.), then also that disturbance of the ray occurs, by which it is broken up into waves of varying intensity, whose presence will be revealed as "polarization" colors, when tested between crossed nicols.

However, exactly how the molecules of certain substances, or within certain structures, produce this disturbance in a transmitted ray—or even how they produce the familiar disturbance in a reflected ray, called "color"—we cannot explain, as long as the very forms and the modes of optical action of the infinitely minute molecules themselves remain beyond our ken.



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# LABORATORY PRACTICE

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# Journal of Applied Microscopy and Laboratory Methods

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## A Method of Removing Chick Embryos.

One of the chief difficulties encountered in the removal and fixation of chick embryos is the wrinkling, curling up, and folding, which occur when the fixative is applied.

Foster and Balfour (*Elements of Embryology*, 1896 p. 442) direct to float the embryo on a glass slide, allow the edges to dry fast and then immerse slide and chick in the fixative. Lee's *Vade-Mecum* recommends that the chick be spread out on the convex surface of a watch-crystal and then immersed. Both of these methods are cumbersome, and it is often difficult to remove the embryo from the slide after fixative without injury. In some laboratories the embryo is pinned out on wax or cork with animal spines and then immersed in the fixative. This often results in distortion and tearing.

I have used the method hereafter described for several years and find it gives such uniformly good results that it seems worth while describing for the benefit of those who are not familiar with it. This method is used in several laboratories and the author makes no claim of originality for it.

Holding the egg, lying on its side, in one hand, crack the shell around somewhat above the equator. Beginning at the larger end of the egg, with blunt scissors cut through the shell and shell membranes completely around the egg, or break the shell and membranes around this line with fine forceps. Care must be taken not to push the point of scissors or forceps too far through the shell or the vitelline membrane will be punctured. The cutting of the shell is attended with less risk to the embryo if the side of the egg which lies uppermost while in the incubator is kept uppermost during the operation, and if a part of the albumen is allowed to run out at the opening made in the larger end of the egg before proceeding with the cutting.

After the shell has been cut completely around, the upper cap of the shell may be removed and part of the albumen poured off, leaving the yolk in the larger part of the shell.

While this method is attended with more risk than the method by which the shell is removed in small pieces from the shell membranes, and then the latter is removed in strips with forceps, yet the saving in time more than compensates for the slight loss of embryos.

The part of the shell containing the yolk is now immersed in a vessel of

warm normal salt solution until the yolk lies just below the surface. The embryo will usually lie uppermost. If it does not, it will revolve to this position, if the chalazare be cut.

With fine scissors *rapidly* cut *completely* around the embryo and so much of the embryonic areas as it is wished to retain. Usually this cut is just outside the area vasculosa. This is the most critical part of the entire operation, for as soon as the vitelline membrane is punctured the yolk contents begin to flow out and mix with the albumen and the embryo tends to revolve in the opposite direction. This latter is best prevented by placing the thumb or finger of the left hand lightly upon the yolk before beginning to cut, thus holding it firmly in place.

Having cut *completely* around the embryo, remove it, together with some of the underlying yolk contents, to another dish of warm salt solution. This is best done with a lifter or a small horn spoon. The embryo may now be separated from the adhering yolk and from the vitelline membrane, either by gentle jets from a pipette or by shaking gently.

Now float the embryo upon a *flat* glass plate (a two by three inch slide is convenient) and remove it from the salt solution. The embryo should be spread out on the glass perfectly flat without folds or wrinkles.

Prepare previously a square of unglazed paper (thin filter paper, ordinary toilet paper, or thin tissue meets the requirements) one to two inches square, according to size of embryo. In the center of this cut an oval hole larger than the embryo and area pellucida. Put this dry paper over the embryo as it lies on the glass plate. The embryonic area will adhere to the paper while the embryo is suspended without any distortion or strain in the hole.

With a pipette put a few drops of fixative on the area of paper to which the embryonic area is attached and let stand for one or two minutes only. In this time the embryonic area will become fixed to the paper, but not to the glass.

Slowly immerse the plate in the fixative when the paper with embryo attached will float off on the surface of the fixative. If filter paper is used it will sink at once, but if non-absorbent paper is used it will continue to float on the surface, and by reversing the slide before immersing the chick may be thus held suspended in the fixative, with no danger of flattening from pressure against bottom of the dish.

After fixation and washing the embryo may be removed from the paper by jets from a pipette or with a thin-bladed lifter. Since the paper serves as a very convenient means of handling the embryo, it is advisable not to remove it until just before clearing. It cannot well be done after clearing, because of the brittleness acquired in this process.

This method gives preparations which are neither distorted, torn, nor wrinkled, and the loss from accidents is very small, since the paper serves as a protective frame for the embryo. I find that it is not convenient to use for chicks under 20 hours, but is available for all older stages.

In making whole mounts, the edge of the embryonic area is often ragged. This may be trimmed to a smooth, circular periphery by placing on glass or wax and cutting with a cork borer of the proper size.

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## The Agar-agar Method for Embedding Plant Tissues.

Some time ago I noticed an account of a method for the rapid fixing and embedding of fresh animal tissues in a five per cent. solution of agar-agar. The method was worked out by Drs. Bolton and Harris of the St. Louis University, and first appeared in *Am. Med.* 5: 838-839, and later in *Science*, 17: 1007, 1903. It occurred to me to try this method on plant tissue. I wrote to Dr. Bolton for particulars of the method, and he informed me that he had not tried it on plant tissue, and sent me a small amount of the prepared agar-agar.

According to Bolton and Harris, "the method consists essentially in placing the fresh tissues in a hot 2 per cent. solution of agar-agar to which 10 per cent. of formalin has been added. The temperature of this fluid should be kept at about 70° C. After remaining in the solution from one to several hours, the tissues are removed and attached to blocks with a 5 per cent. solution of agar-agar containing 10 per cent. of formalin. The heat and the formalin harden and fix the tissues at the same time the agar-agar impregnates it. After fixing the tissues to blocks these are placed in 95 per cent. alcohol and allowed to remain from two to four hours, and the tissues are then ready to be cut into sections, which can be stained, cleared, and mounted on slides in the usual way employed for celloidin sections."

The following are some of the plant tissues on which the agar-agar method was tried: Young ovules of water lily showing the embryos; cross sections of geranium stems; cross and longitudinal sections of begonia stems; cross sections of young ovaries of canna and fig; cross section of the leaf of *Ficus elastica*; cross section of the sori of fern leaf; longitudinal section of petiole of Boston ivy; cross sections of stamens of canna; longitudinal section of moss plant (*Bryum roseum*); cross section of leaf of *Asimina triloba* showing *Phyllosticta asiminae*; cross section of raspberry leaf showing rust.

At first the 5 per cent. solution of agar-agar was used for the fixing and embedding of the tissues. Later the 2 per cent. solution was used for the fixing and found to be more satisfactory.

The 2 per cent. solution of agar-agar can be made as follows: Take 10 grams of agar-agar to 500 c. c. of distilled water and boil for two hours. Then pour the hot solution into a high cylinder and allow it to cool slowly until the cloud has fallen. After the solution has cooled, cut off the clear upper portion and put it in a glass jar. Place the jar in a basin of water and heat it until the agar-agar is melted. Then add formalin in the proportion of 1 part of formalin to 9 parts by volume of the melted agar-agar.

The 5 per cent. solution is made in the same way as the 2 per cent., only 25 grams of agar-agar to 500 c. c. of distilled water are taken. Formalin should be added in the same manner and proportions as in the 2 per cent. solution. The 5 per cent. solution when melted is quite fluid, but when cold it is about the consistency of new cheese. It becomes much firmer on the blocks after exposure to the action of strong alcohol. Large quantities of the agar-agar solution can be prepared and preserved in air tight vessels to prevent evaporation.

For fixing and embedding only a small amount of the agar-agar solution need to be taken. The solution should be kept at a temperature of 70° C. The fresh tissues are first placed directly into the hot 2 per cent. solution and let remain for about two hours and are then transferred to the 5 per cent. solution and let remain for one hour or more, when they are ready to be embedded. The tissues are embedded on wooden blocks. With a small camel's hair brush put a layer of the hot agar-agar on one end of the block, let it cool for a few seconds and then place one of the pieces of material on the block. Cover with more of the agar-agar solution until properly embedded. After fixing the tissue to the block, place in 95 per cent. alcohol and let remain for twelve hours. The longer the agar-agar remains in the alcohol the tougher it becomes. The writer left embedded material in alcohol for six weeks without any noticeable injury. The material is sectioned on a sliding microtome the same as in celloidin sections. The knife must be very sharp, as the sections are somewhat friable, and kept moist with 95 per cent. alcohol, and likewise the blocks, during the sectioning. The sections can be stained the same way as celloidin sections. Safranin and gentian violet, and Delafield's hæmatoxylin are favorable stains.

It seems that this method will be especially valuable in the study of rusts and other parasitic fungi where it is advantageous to make microscopic sections. The *Phyllostictia*, mentioned above, was collected in October and was the late infection. The tissues of the leaf were entirely dead. The infected parts were cut in small pieces and placed directly in the hot 2 per cent. solution of agar-agar for two hours, and in the 5 per cent. solution for an hour, and then embedded. The sections obtained were cut at 20  $\mu$  and showed the conidia and spores in fine condition. The rust on the raspberry was collected in September, 1902, dried and kept in the herbarium. The material was very firmly pressed, thoroughly dry and brittle, but in spite of these facts the sections were as satisfactory as in the fresh material. The delicate peridium was sectioned without any injury and the hyphæ could be seen in the adjacent tissues of the leaf. The parts sectioned were placed in warm water about 70° C. and let remain for two hours, then in the hot 2 per cent. agar-agar solution for two hours and then in the 5 per cent. solution for an hour and embedded. The sections were cut at 20  $\mu$ . The sections containing the fungi were taken through the alcohols to water and stained in a weak solution of safranin, then washed and mounted in glycerine.

This method is applicable where a histological study of the plant tissue is desired, but does not seem satisfactory for cytological work. Sections were cut at 20  $\mu$  in thickness, but the most were cut at 25  $\mu$  and 30  $\mu$ . The best feature of this method is that it is simple and quick. The aqueous solution of agar-agar at once penetrates the tissues without any preliminary dehydration.

## Industrial Microscopy.

### II.

#### APPARATUS.

The first to be considered under this head is the microscope itself. The compound microscope is an instrument consisting of two combinations of lenses supported in place by a tube of metal. For the adjustment of the system with respect to the object the tube is controlled by some form of device for coarse movement and, usually, some form of micrometer movement by which the whole system is accurately moved through short distances.

The combination of lenses nearest the object is known as the objective, while the set nearest the eye of the observer is called the ocular or eyepiece. The objective projects a real image of the object at a distance back of the lens. The exact location of this image depends upon the relationship between the focal length of the objective and its distance away from the objective. When in proper relationship to the object a real image of it is brought to a focus in the plane of the diaphragm of the ocular by means of the objective and first lens of the ocular combination. The upper lens of the eyepiece treats this image as a real object, magnifying it again according to its power.

There are at present so many types of instruments upon the market that the final choice must be made by the worker himself in view of the work to be accomplished and the outlay he has at his command for the purpose. A few general suggestions may perhaps not be out of place at this time, however. Through the courtesy of the Bausch & Lomb Co., the writer is enabled to show in Fig. 1 a cut of one of their make of instruments embodying many of the features mentioned further on. For many lines of work a simpler type would be found adequate. In purchasing an instrument for general work a stand should be secured allowing the use of substage condenser, polarizing apparatus together with the selenite disk, and furnished with ready means of controlling, by means of a suitable substage diaphragm, the cone of light entering the objective.

The lenses on an instrument should be so chosen as to be capable of giving a range of magnifications from 60 to 300 diameters for ordinary work, though a lower power (about 30) is often useful as a searcher and in use for measuring fibers or other long bodies. On the other hand, for some small objects such as some of the starches a power of 500 is useful. Such combinations can of course be secured by consulting the catalogues of the makers of microscopes.

But magnification is by no means the only or even the most important requisite in a microscope. The power of resolution and definition are without doubt more important. Many times a lens of comparatively low power has been found more serviceable than one of higher power, for the reason that it had the better defining power. Flatness of field, numerical aperture and achromatism are important factors to be considered since they enter into determining the defining and working value of the lens.

Many *interesting* features may be seen through almost any lens, but when it

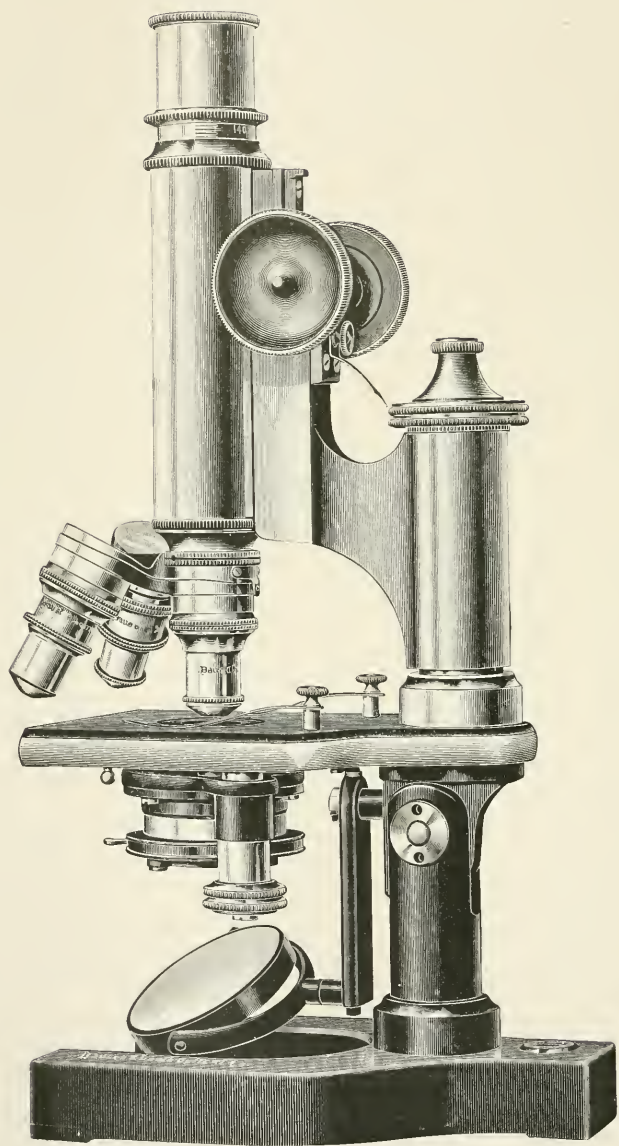


FIG. 1.



comes to determining some faint, though nevertheless distinctive, structures on certain materials which the microscopist has to examine, the best are found to be none too good.

The achromatic series of objectives are, on the whole, very good, though at present the series par excellence is without question the apochromatic; the greatest drawback to them from the standpoint of the purchaser being the fact that, for corresponding powers, they are from two to four times as expensive as the achromatic. If the achromatic objectives are finally chosen, the Huyghenian oculars will be satisfactory; but for the apochromatic series the compensating series should be used. This is necessary, since, as the name indicates, they compensate for certain overcorrections of color made in the present series of apochromatic lenses.

To test the defining power of an instrument it is usual to examine such objects as starch from potato or bean, diatoms and scales from a butterfly's wing or other objects possessing delicate markings or structures. Each of these objects possesses well defined markings, which with proper conditions of light should be well shown by an instrument intended for exact work.

The starches possess rings, usually concentric in the bean or eccentric in the case of the potato. For examination small samples are mounted in water or dilute glycerine. On account of the transparent nature of the grains the substage light must be cut down by the substage diaphragm until by trial the most exact detail of markings is obtained. With a good instrument the outlines of the grains should appear sharp and distinct with no (or at least very little) color. Bright colors of course will not be expected since they usually take the form of a faint suggestion of blue or yellow around the edge of the object, depending on which side of the focal plane the grain happens to lie. Hence, by changing the conditions from one side to the other by focusing, the colors may be seen to alternate.

According to the writer's experience this phenomenon is also ordinarily accompanied by the grains appearing to be covered by a slight fog or haze. The latter may, however, be caused by soiled lenses, or because that upon one or more of the lenses there has been deposited some form of film.<sup>1</sup> The feature of non-achromatism in low or medium power objectives can be strikingly shown by placing a fine wire gauze or other opaque object having openings at short intervals over the surface, in the field and focusing upon it. In this condition the colors will be strongly shown if the lens is not accurately corrected.

In a similar manner to that described for the starch, examine also the butterfly scales and diatoms. The former is characterized by having longitudinal markings upon the surfaces and also (in some species at least) fine connecting lines. The diatoms show various kinds of markings, depending upon the species. One form is shown in Fig. 2.

In using the microscope when focusing upon a new specimen it is a matter of wise precaution against the accident of thrusting the objective forcibly down upon the slide to lower the objective nearly to the cover-glass, meanwhile watch-

<sup>1</sup> Edward Bausch, "On Determination of Supposed Defects in Microscope Objectives." JOURNAL APPLIED MICROSCOPY AND LAB. METHODS, Vol. I, No. 1.

ing the operation carefully with the eye almost on a level with the lower edge of the objective. With all ordinary objectives, except those of high power, this will bring them too close to the object. Then observing through the ocular slowly raise the objective by means of the coarse adjustment until the object comes into view. Then lastly with the fine adjustment bring into exact focus the portion desired.

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(To be Continued.)

## The Technique of Biological Projection and Anesthesia of Animals.

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### XIX. DIRECTIONS FOR MOUNTING LIVE ORGANISMS IN GLASS CELLS.—Concluded.

#### C. Mounting animals in open top cells.

##### *The demonstration of the circulation of water through colonies of spongilla.*

This experiment not only illustrates in an interesting way a life phenomenon exhibited also by marine sponges, but shows the sensitiveness of the sponge cells to heat and their contractility. Colonies of *spongilla* frequently have their oscula at the ends of very delicate and nearly transparent tubular extensions of the body substance beyond the spicules. These tubes may be seen with a hand magnifier on examining in sunlight a submerged colony which has not been disturbed for some time.

To demonstrate, by projection, the outflowing current of water and the sensitiveness to heat of the cells composing the oscular tube, take all of a small colony, or as much as possible of a larger one, and transfer it on its natural support, i. e., on the twig of green plant or fragment of the wood on which it has grown, to an open top cell (Fig. 9, No. 14 or 17). If the transfer is made quickly so that the colony does not dry off at all, the tubes will soon be found to be normally expanded. Fasten the colony in place in the cell either by blocking up or, in No. 14, by the use of the movable partition, spring and wedge so that the tube will appear in profile extending outward from the surface of the colony when projected on the screen. Use a low power objective. To make the outflowing current from the oscula most evident, place in the cell a few very small pieces of decaying leaves from an aquarium, or any other small objects which will neither float at the surface or quickly sink to the bottom of the cell. These floating fragments are drawn slowly to the osculum, are caught and sent whirling around in a vortex caused by the steadily outflowing current.

Under the heat stimulus which the intense light carries with it, the delicate, almost transparent oscular tubes are seen to contract slowly in diameter and length; but they will again expand to their normal size, if the exposure to heat is not too intense or long continued.

Mounts of *spongilla* in a relatively large amount of water may often be kept in good condition for two days or more, and *hydra* in the open top cells live for several days.

*The demonstration of all the successive stages in the metamorphosis of an insect with living specimens.*

This offers an interesting and instructive series of experiments. One of the best species with which to do this is the mosquito. Eggs, larvæ, pupæ, and imagos are readily obtained during the warm season, and in winter may frequently be found in warm greenhouses, where the eggs are laid in barrels of liquid fertilizer after the heat due to the fermentation process has subsided. Egg masses should be mounted for projection in hollow-ground cells. Larvæ and pupæ may be collected with a fine mesh wire strainer and mounted in clear water in an open top cell. When projected on a screen with low power objective, their peculiar wiggling, the elevation of their caudal spiracles to the surface for the purpose of respiration, the cleaning of this spiracle with the mouth parts when it has become clogged in any way, and the ordinary movements of their mouth parts in feeding are easily demonstrated. To quiet them so that more detailed studies may be made with higher power objectives, add to the water in the cell two or three times its volume of one per cent. solution of chlorotone. At first the larvæ become very active, then gradually cease wiggling and only the mouth parts are actively moved, and soon the animals fall to the bottom in a completely passive state. By diluting the anesthetizing solution with water the semi-passive state may be prolonged, and this has been found to be a most favorable condition for the study of the mouth parts. If not subjected to the action of a strong solution of chlorotone for too long a time, the larvæ may be revived by withdrawing the anesthetic and adding clear water. Adult mosquitoes may be projected alive when mounted in a life box or glass cell as described below, but they are too delicate to endure the heat from a strong light for any great length of time. For temporary mounts they may be placed in water in hollow-ground cells so as to give dorsal, ventral, or lateral views.

Open ring cells (Fig. 9, Nos. 18 and 19) are apt to leak water over the top as a result of capillary action; but it may be stopped by smearing the upper or cut edge of the ring, especially at its points of contact with the slide and cover-glass, with a very small amount of vaselin or paraffin.

D. Mounting live insects, e. g., flies, mosquitoes, bees, grasshoppers, and crickets, in air in life boxes and glass cells (Fig. 9, Nos. 10 to 12).

Many of the larger and stronger species of insects are easily held in the hand and placed in the box and quickly covered. Delicate species and those which are apt to sting may be caught in a wide mouthed bottle or under a beaker. To transfer them to the cell place a card over the mouth of the bottle or beaker, with a pen-knife cut a hole in the card a little smaller than the diameter of the cell, place the cell or cover of the life box over the hole, and induce the animal to pass through either by shaking or holding toward the light. As soon as the animal has passed into the cell slide it along the card to prevent its returning to the beaker. Next place the cover of the cell, or bottom of the life

box, on the opposite side of the card and carefully slip the card out from between them, and the insect is securely enclosed. The cover of the life box may be slipped down on the insect so as to hold it in one position if desired; but the cover of the cell should be held in position by rubber bands arranged close to the edge, and the insect is able to walk, or even to attempt flight, in the cell.

Organisms of any kind, when mounted in air as above described, cannot endure as intense a degree of heat without injury as those mounted in water. As a rule, therefore, it is necessary to place them in the light at such a distance from the principal focus of the condensing lenses that the heat will be moderate and the light sufficient for the low power objectives which the depth of the cell and size of the organism require. Much excellent work is possible with no higher power lens than ordinary quarter or half-size projection lenses used for lantern slides. A common house-fly may be mounted in a life box and projected with a one-inch objective. With an enlargement up to from six to ten feet in length, it is an interesting and instructive object, as it exhibits its characteristic motions of legs, the cleaning of the wings, and action of the mouth parts.

## XX. DIRECTIONS FOR MAKING AND USING LARGE COMPRESSOR-CELLS.

Animals having more or less cylindrical bodies, e. g., earthworms and leeches, need to be flattened by compression in order that the details of their anatomical structure may be clearly seen under the microscope while they are alive and have not been dissected. In an earlier article in this series, No. X, Jan. 1903, a simple gravity-compressor for use on a horizontal microscope stage was described and illustrated. For use on the vertical stage of a projection microscope and whenever more pressure than that produced by gravity is needed, a compressor-cell (Fig. 9, No. 15) is useful. This compressor-cell is not on the market in its complete form, but is easily made. As used by the writer, it consists of a compressor (B. & L. Opt. Co. No. 1264) with the following additions: A spiral spring is placed on the screw at the fixed end of the metal plates for the purpose of keeping the upper metal plate up against the screw when the plate is swung around to permit the removal or introduction of the glass plates. This spring is not absolutely necessary, but it increases the ease and speed in handling. To convert the compressor into a cell of adjustable depth, thin sheet rubber, such as is sold for repairing inner tubes of bicycle tires, is cut to the size of the glass plates and about one-fourth of an inch wide. These gaskets are cut through on one of the long sides to allow any excess of liquid to escape during compression of an animal. In place of the gaskets a soft rubber tube may be drawn over a small copper wire and then bent into the form and dimensions of the glass plates, but this arrangement is not as adjustable as that with gaskets.

To mount a worm in the compressor-cell place the animal on the lower glass plate and build up on the plate as many gaskets as necessary to prevent too much compression when the screws are tightened. With earthworms the total thickness of the gaskets should be a little more than half the diameter of the specimen. Fill the space within the gaskets and around the worm with water, or chloretone solution if the worm is anesthetized, and place the upper glass plate in position. Adjust the upper metal rim and tighten the screws equally so



as to compress the worm and prevent leakage from the cell. By twisting an earthworm slightly when arranging it on the compressor one end may be made to give a dorsal, the other end a ventral, and intermediate parts a lateral view, so that all anatomical details may be made out in one mount. The finest results are obtained by selecting small earthworms having transparent body walls through which the blood shows a pinkish color, and feeding them on moist clean filter paper in a battery jar for a day or two to clear the intestine.

To those who are not familiar with the results rapidly and easily attained in the study of live earthworms by the combination of anesthesia by means of chlorotone with a compressor to flatten the animal slightly, the following summary will seem to be an exaggeration; but it is a list of anatomical details distinctly seen with the compound microscope in specimens mounted and studied by students in the second year of the high school course. The structures made out with such clearness as to be identified with certainty include the following: in the body wall—the setæ and their muscles, the longitudinal and circular muscles; in the cœlom—the cœlomic fluid and its corpuscles, the septa, and the parts of the alimentary, circulatory, and nervous systems; in the alimentary system—the buccal sac, the pharynx, œsophagus, crop with its contents, gizzard with its contained food and sand grains being moved around by the muscles seen in the wall of the organ, the intestine exhibiting peristaltic motion and its covering of chloragogue cells; in the circulatory system—the pulsating dorsal vessel and five pairs of “hearts,” circular vessels, sub-intestinal, sub-neural and supra-neural blood vessels, and the openings of vessels leading from the underside of the dorsal vessel into the typhlosole; in the nervous system—the ganglia and commissures of the ventral nerve chain, the pharyngeal collar and branching nerves, and the cerebral ganglia; the nephridial tubes are readily seen, the funnels are more difficult, and the cilia in the mouth of the funnels are still more difficult to see in action, but they have been seen clearly. While the above list enumerates the details studied with a compound microscope in the hands of young students, more than half the number have been studied also from the screen both in class room and in public lectures.

Directions for demonstrating three important phenomena of the circulatory system:

- A. Circulation of the blood as seen in the capillaries.
- B. The pulsation of the auricles and ventricles of the heart.
- C. Valvular action in the heart and the movement of blood through it.

All three of these phenomena cannot be shown in the same animal, or even in different members of the same species; but each may be demonstrated in animals of two or more species. For the capillary circulation use the tail of a fish or tadpole, or the web of a frog's foot; for the pulsation of the auricles and ventricles, several species of fresh water clams; and for a view of the moving valves of the heart and the rapid inflow of blood, its sudden pause and equally quick rush into the arteries, *Daphnia pulex* and especially the nymphs of the dragon-fly (*Agrion*) may be used.

A. The circulation of the blood in the capillaries is more easily demonstrated in the tail of a tadpole than elsewhere for two reasons. First, the tad-

pole is more easily handled and, second, its blood corpuscles are large and the capillaries are correspondingly large. The method of anesthetizing tadpoles and of mounting them for the projection of the circulation of the blood with a projection lens such as is used with lantern slides will be found in Art. XIV (May, 1903) of this series.

For projection with one-quarter to one-inch microscopic objectives, the anesthetized tadpole is mounted in a life cage (Fig. 9, No. 14), care being taken to have as much as possible of the animal's tail in contact with the inside of the front of the cage so as to be within the working distance of the higher power objectives mentioned above. Focus on the thinner parts of the tail for the capillaries, and on the thicker portion for arterial and venous trunks.

A. H. COLE.

## The Museum.

### XII.

#### ACCESSORIES AND LABELS, ETC., ETC.

Besides the cases which form the larger receptacles for objects, the museum curator is compelled to consider various instrumentalities for arranging his specimens both in and outside of the cases, besides the very trying questions of the security, the curing and storage of specimens.

#### DRAWERS.

There is a need in every museum of drawers, and with them some sort of interchangeable system. Drawers under the flat cases of an exhibition hall are

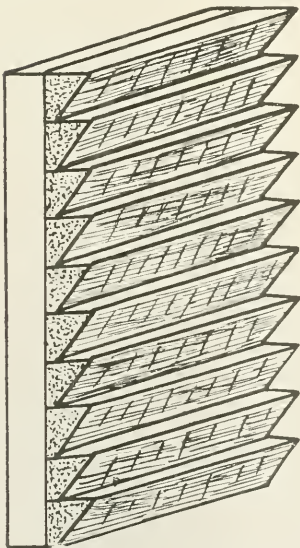


FIG. 83.—Drawer Runways.

very convenient, and readily permit of the storage of overflow specimens, of specimens less well adapted for exhibition, and of the interchange of specimens exhibited, with others which are suitable for exhibition, but which cannot, for lack of room, be all exhibited at once. Such drawers should be accommodated to a system of runways or slides stepped up into stacks (Fig. 83), and the unit drawer being fixed its depth should be equal to, or a multiple of the height of the runways so that the top of the drawer will be on a level with the next succeeding runway. Every other drawer in a stack can always be varied in depth so as to be a multiple of the depth of the unit drawer, or indeed any multiple of the height of the runways.

The dimensions of the unit drawer, as designed in the National Museum, is 24 by 30 inches. In different departments, however, and

in different rooms of the same department the unit drawers may vary in size ; but it is obvious a controlling advantage to be able to take out a drawer from any part of a museum and find it fit the stacks in any other, even remote parts, of the same museum. Such uniformity can, however, scarcely ever be thoroughly attained.

As drawers will contain specimens in many departments, Mineralogy, Palaeontology, Oology, Archæology, which are placed away in paper trays, it will be found very useful to establish an exact relation between the size of the drawer and the size of these paper trays, so that the latter will, from back to front and from side to side of the drawers, compactly fill the latter. This prevents shifting and dislocation in pulling and pushing the drawers out or in. It is inadvisable to use drawers under cases that do not fit closely together, and depend, for their



FIG. 84.—Cases of iron fronted drawers.

extrication, upon a space left between them. Such openings are only another invitation to dust. Bring the drawers *closely together* and put a handle on each drawer-front. As an additional protection to their contents, enclose a section of drawers, under a table case, by two or one outer door, which shuts upon them, and forms a sensible barrier to intrusion, whether inorganic or human. The under drawers of flat cases have already been shown in preceding figures. As a protection to large stacks of drawers in storage, duplicate, or study rooms, the drawers may be encased in iron boxes, and each drawer have an iron front. Such stacks have been in use in the New York Museum (Fig. 84). Practically this may not generally prove efficacious at all in the accident of any extended conflagration, but it seems reasonable to expect from it some security in the case of a local fire. The fronts of such drawer stacks can be readily washed, and defacement by cutting, writing, etc., is impossible. When any stack of drawers,

either under a flat case or in the higher tiers, in storage and duplicate rooms, is shut in behind an outer door, it is often convenient, especially in the study rooms, to make this outer door a glass door, so that labels and descriptions of contents on the drawers behind can be read without the annoyance of opening the door itself for such a purpose.

The painted tin-can for the preservation of study collections in ornithology and mammalogy is important. Their use is of course general. They consist of a box with an upper edge provided with a gutter into which the cover fits, pressing down upon a rubber strip at the bottom of the gutter, thus insuring air-tightness. The dosing of the contents from time to time with bi-sulphide of carbon,



FIG. 85.—Tin cans for preservation of skins, etc.

in conjunction with this hermetical seal, seems to provide an indefinitely long (Fig. 85) life to very perishable objects.

Turnstiles are invaluable adjuncts to cases, and for some objects or exhibits are absolutely indispensable. In the British Museum such turnstiles contain the mounted specimens of local herbaria, and Dr. Britton will put them to an identical use in the Museum of Botany in the Botanical Gardens in New York. For photographs, illustrative plates—as once employed by Pres. Jesup for his wood collection—they form the only convenient means of accommodation for a large series of planular objects. They should be simple and strong. An effective turnstile designed by the writer is shown in Fig. 86. The central part is on ball-bearings, enclosed in the box A, so that the visitor can at will revolve the post



and bring the object examined into the best light. Many flat objects can be mounted in frames on walls, and a very notable effect could be produced by arranging the wall space of *narrow* connecting halls for the exhibition, in this way, of vertebrate fossils, slabs of crinoids, etc. The frame work should, if possible, relieve by contrast the color of the objects. Figure 87 shows an attempt at decorative effect gained by an arrangement of reindeer skulls on wooden shields, uncased, on the wall of a stairway. In this case an explanation of the objects is printed on the wall itself beneath them. The method is, to the mind of the writer, hardly wise. The use of walls in large museums, in this way, as sign boards or label stations is somewhat provincial and dwarfing. It seems generally a better practice to frame or encase all such objects, if placed on the walls, and a certain amount of abstinence must indeed be observed in museums of some architectural pretense as to crowding wall spaces with too many objects at all.

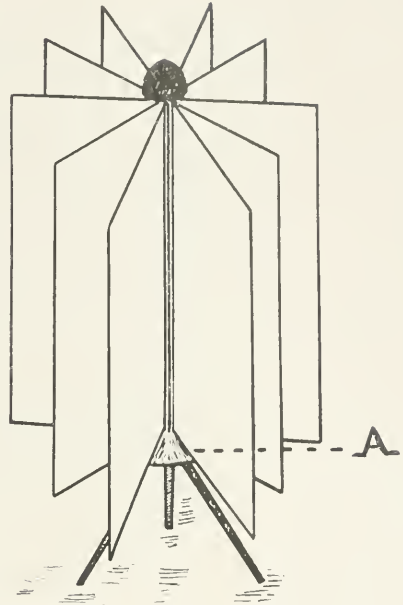


FIG. 86.—Turnstile for photographs, charts, etc.

The many contrivances by which objects of various sorts are held in position, or the numerous receptacles for them, and the manner in which they are labelled, constitute *paraphernalia*. Trays, labels, supports, stands, pins, blocks, plaster cells, rods, backings, covers, etc., etc., all make up paraphernalia.

#### TRAYS.

More diversity than would be considered probable, may exist in trays; their sizes, heights, color, and attachments all offering points of difference. A form of exhibition tray has been in use in the National Museum, for a long time, which receives some praise, but can hardly be recommended with enthusiasm. These are made with rather high sides and with a bevel front, upon which the label of the specimen in the tray rests. They are black, and may have the bottom covered with paper or a colored fabric. In the British Museum the minerals are laid on jeweller's wool, which is packed into the edges of a rabbeted block whose edges form a block frame. This method, for the purpose, has met with unqualified approval. A similar or identical effect can be attained by covering the bottoms of the shallow paper trays with jeweller's wool, which is fastened down by very thin black strips of black wood (painted and shellaced) fitted inside the tray (Fig. 88). The cotton rises or puffs slightly, the frame of black gives individualism and elegance, and the effect is very attractive. In these cases the label is fastened to a sloping block placed within the tray, so that the edges of the trays come in close contact. Such trays are made in an

ascending series of dimensions, the longer side of one tray forming the shorter side of the tray next above it in size, as 2 x 3, 3 x 4, 4 x 6, 6 x 8, 8 x 12, etc., with the occasional use of square trays and odd dimensions. These trays have sides three-eighths of an inch in height. Their corners are stiffened by small wire elbows, hidden in the cardboard.

Plaster of Paris trays with bevelled edges have been applied to the exhibition of shells, but with poor effect, their white porous surfaces absorbing dirt and dust, and soon showing a sullied and repulsive surface. Porcelain trays have also been suggested, but they are expensive, and present a cup and saucer tea-service effect which is slightly ludicrous. Dr. Schuchert, of the National Museum, has put in use a terra cotta tile for holding fossils, but its results are doubtful. The best or most attractive method of exhibiting fossils has yet to be



FIG. 87.—Reindeer heads on stairway, A. M. N. H.

discovered. An attempt will be made to solve this by the use of backgrounds and vertical screens for the tabular pieces, containing fossils, while the detached individual specimens can be arranged in trays or on boards of strikingly contrasted color, as ebony or ivory white (Fig. 89).

Trays should not be too deep, simulating boxes; their sides should not exceed one-half an inch, and that usually is too high.

#### LABELS.

Dr. Goode has drawn the attention of museum curators and authorities to the importance of labels, and in his report on the National Museum has feelingly expounded the whole subject. It really does seem that the critical position of the label writer is somewhat overstated, and the extreme altitude of lexicographical excellence assumed for him rather exaggerated.

But with that we are here hardly concerned. The technique (this section) of labels, involves their colors and disposition; their contents, size of type, etc., fall under the later sections of System and Effect.



FIG. 88.—Paper trays rimmed in black holding cotton for minerals, A. M. N. H.

A blue-gray label has been, for a long time, used in the American Museum. It is perhaps unsatisfactory, though as a color exceedingly good, in spite of a certain coldness. The buff tints seem preferable. They do not fade, and are a warmer tint. In the mineralogical cabinet of the New York Museum red ink on a gray ground has a striking effect. These labels have been called *Rubrics*.

Besides these colors, Royal Wooster, maroon, brown, various grays, black with gold or silver letters for large labels, have been adopted. Large outside labels of thin mahogany board with gold lettering are admirable. A label, for separate cases, of black ebonized wood, with gold letters, is excellent and effective. Dark brown leather labels with gold letters are also attractive, and can be



FIG. 89.—Fossils on cardboard, A. M. N. H.

used to distinguish important gifts. On the whole, in the cardboard labels on the inside of the cases the plain border label is to be preferred to the label with a line around it. It is more chaste. But, if the expense and labor can be afforded, the cardboard label sunk into, or attached to, a black or mahogany strip of wood, so large as to make a frame around it, is very elegant. The larger general labels in cardboard should all be framed in narrow bead frames of wood. The outside wood framed labels of black or mahogany, if on wall cases, are attached by picture moulding hooks, and if on desk cases are supported by upright short brass rods. A simple device for raising a label in a tray is to push two pins into the front side of the paper tray, and rest the paper label on them. Short or longer thin wooden standards, fixed in square or round bases, with a split apex into which the label is inserted, afford useful and of course very mobile labels inside of cases.

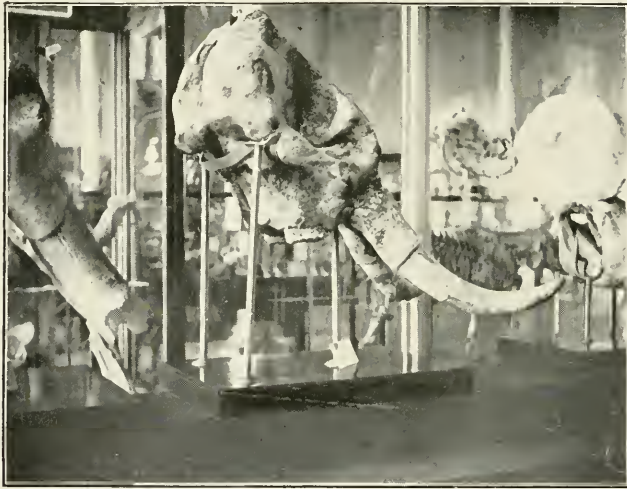


FIG. 90.—Fossil skull on brass rods, A. M. N. H.

#### SUPPORTS, STANDS, BLOCKS, PINS.

To be clearly seen many small crystals and sometimes small shells demand a support, which lifts them into individual prominence, while large groups of crystals and coral masses, as well as all taxidermical specimens, need stands and pedestals as an artistic embellishment. Small black pedestals of wood can be bought from dealers which will serve for mounting crystals, upon which the crystals can be attached by black wax. Glass rods are also in use, and frequently shells or other flat objects are attached to them by wax. A rather pleasing system, which admits of some variation, is to take a plate of glass, paint it carefully on one side, as a background, and attach to it shells or sections, by wax. These plates can be used in synoptical cases and are a cheap and useful device where wall or flat vertical spaces are to be used up. In the mounting of skulls and the large fossil remains of the mammalia from the West, Mr. Herman



has used with splendid results, brass rods socketted in mahogany blocks (Fig. 90). For mineral masses, stands of ebonized wood or of mahogany are superior (Fig. 91), but oak, chestnut and black walnut can be favorably used. On steeply slanting shelves the label block can be used as a support to the object, or, in the case of large shells, a V-shaped collar. Devices are innumerable for meeting such problems, and the skill and taste of the exhibitor can be indefinitely exercised.

A pin is a form of support, whether it transfixes an insect, or holds up the end of a chinook blanket, or restrains a clam shell from sliding off an inclined board, or exhibits, poster-like, a label, or more differentiated still, with three clamps, grasps a gem. Pin, therefore, is a generic term for metal appurtenances so modified as to meet these different uses. Rods run along the tops of cases, form a convenient rack for the suspension of pictures, maps, etc. The rods are held by staples in the roof of the case.

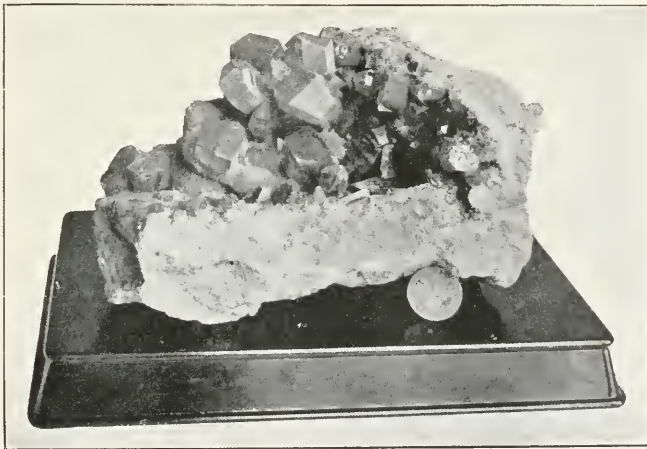


FIG. 91.—Sulphur from Cianciani, Italy.

#### JARS, PLASTER AND GLASS CELLS.

The plaster cells of Denton for lepidoptera are probably one of the most notable inventions in museum installation proposed in the last ten years. They consist in a white plaster block, with a depressed pit holding the body of the insect, whose wings are outstretched, the whole sealed in by a glass cover. Glass cells for food products, medicines, herb preparations, etc., can be formed from five glass plates cemented together by soluble glass, and covered by a glass slide moving on vaseline. A more elaborate form consists of four glass plates socketted in wood, with a glass cover held on by metal pins, which pass through the glass, and can be unscrewed, their ends being driven in the wooden frame at the bottom of the cell.

Glass jars for alcoholics should almost invariably be flat with black painted backs, so that the bleached or diaphanous objects, contained in them, can be clearly seen. Flat glass jars are expensive, but it seems likely that the less munificent managements can make their own jars from glass panes held together

by a water proof cement, buttressed possibly at the angles by a mixture of hydraulic cement and plaster of Paris. The Elliott glass covered boxes holding cotton, upon which insects or plants can be impressed, are a cheaper substitute for the Denton cells and not unworthy. Inverted bell-jar bottles with the stoppered mouth underneath for deliquescent minerals, salts, mineral industrial products, etc., have been long in use, while for almost all such contingencies, the catalogues of the Kny Scheerer Co. (225 4th Ave., N. Y. City), and of Eimer and Amend (18th St. and 3d Ave., N. Y. City), will be found suggestive and adequate.

#### BACKINGS, COVERS, ETC.

Backgrounds may be made to play a most important part in the exhibition of specimens. The contrast of colors between the object and the surface on which it is displayed heightens the charm of museum installation. Backgrounds can

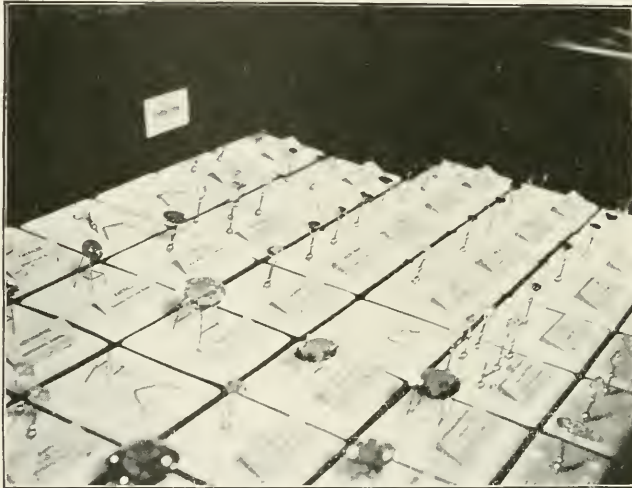


FIG. 92.—Gems on velvet pads, A. M. N. H.

be painted on surfaces or fabrics. Painted surfaces are blue grays—the color so lavishly employed in American Museum in New York, and, for most purposes, very effective—or buffs, even reds; while in fabrics black for white objects, as corals, and green for shells, and an ivory white or maroon for fossils, are a good general selection.

Velvet covering cork tablets have been used for gem collections, the gem being laid on the velvet pad, or fixed into the underlying cork through the velvet, by a pin. This is quite attractive, though perhaps an olive green would, as one color, better replace the patchwork of white and black (Fig. 92). The installation of gems, so as to bring out their peculiar beauty, is not completely solved. The writer has used with excellent results, in gem cases, a *celluloid label* with black and red printing, the black and red lines alternating, as in book titles. These give distinction to the cases, and have a striking and ornate appearance.

In a gem room the elegance of the cases may be emphasized, and some con-

cession made to variety and contrast. The installation of gems, indeed, is an artistic question, and with very handsome collections, may be advanced to a higher note of beauty and elegance.

It will be found attractive in gem rooms to provide small square or oblong table cases, rather deep (8 to 12 inches), and arrange a different gem stone in each, cut material being placed in front, or to one side, and the uncut at the back, or opposite side. Uncut material may also be placed in upright cases above the flat case containing the cut stones. The finished and carved vessels of quartz, jade, fluorite, rhodonite, jasper, heliotrope, etc., etc., should be grouped in special cases with glass superimposed shelves, so that all sides can be inspected. If cut objects of this sort are exhibited in wall cases, the backs of the cases should be mirrors.



FIG. 93.—Transparencies in window, A. M. N. H.

Gem rooms should have electric alarms, and be protected by strong iron gates, with the provision of a constant watchman.

The use of transparencies in windows illustrating geology, mineralogy, ethnology, etc., is most admirable if not pushed so far as to be an obstruction to the illumination. They may be put into wooden frames, and if of the same height held in place by cross bars of wood attached to the sash mouldings. They may also be held in place by elbow irons screwed to the sash of the window and screwed to the frame of the transparency (Fig. 93).

In cases where very small objects, as little shells, minute crystals, intricate *orfèverie*, illuminated missals, or sometimes photographs, etchings, etc., are exhibited, some adjustment should be adopted for bringing the specimens nearer the glass of the sash. The most simple device, and one entirely adequate, is to put in a flat case compartment a false bottom of the exact size of the compartment. Again, some of the small objects amongst the larger ones can be raised upon pedestals. A very excellent and helpful feature, and one which is hardly

ever used, though the author has in vain suggested it, in flat cases, or indeed in wall cases, is to fix over the object, to be closely examined, hand glasses of low magnifying powers. The value of these is considerable, and the pleasing interest evoked by seeing the magnification a sensible attraction to visitors.

I have alluded to turnstiles for the mounting of photographs. A system of surprising efficiency for registering and keeping photographs easy of access is adopted at the Pratt's Institute in Brooklyn. It consists in deep drawers of the depth of the photograph so that the latter can be placed on end like cards. *Separators* made of thin wood or cardboard, labelled across the top, with the name of the country, town, city, district, locality, etc., placed between them, afford, like a card catalogue, instant guidance to the photograph desired. It is a most simple and elegant method of preservation and use.

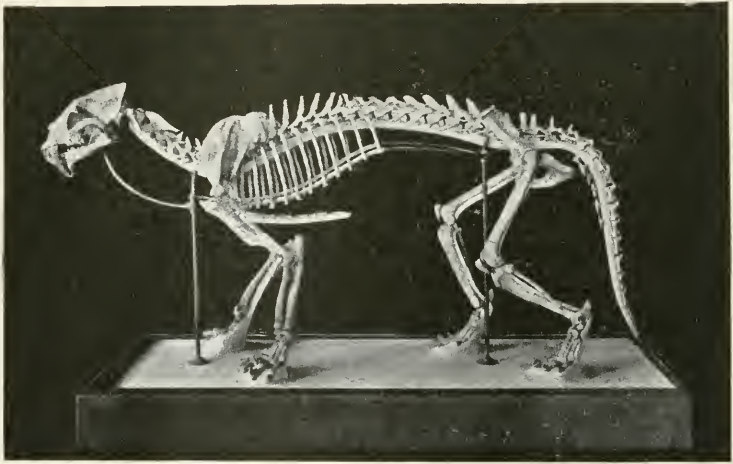


FIG. 94.—Sabre-tooth tiger from South Dakota.

A very remarkably beautiful material for stands has been shown to the author by the agent of Albert Böhm, Wool Exchange Building, New York. It is a very dense black slate susceptible of a superb glossy finish. Its use in trade has been for switch boards, electric attachments, blocks, etc., but there are great possibilities in it as material for pedestals, bird stands, etc. It does not seem to be too expensive and there can be only one opinion as to its beauty. It is imported, already cut and finished, from Austria.

As an example of very beautiful installation, the plate (Fig. 94) is given of a sabre-tooth tiger from South Dakota, prepared by Mr. Hermann, in such a way that every bone can be removed for examination. L. P. GRATACAP.

American Museum of Natural History.



## Laboratory Outlines for the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

### SUBKINGDOM, PTERIDOPHYTA (HOMOSPOROUS).

A series of ferns to illustrate the evolution of complex organs from simple ones.

LV. *Ophioglossum vulgatum* L. Adder's-tongue.

Class, Filices. Order, Ophioglossales. Family, Ophioglossaceæ.

This simple fern may be collected in spring and summer in moist meadows and thickets. The entire plant should be dug up and care taken so as not to injure any of the roots. Preserve in 70 per cent. alcohol and press some for herbarium specimens. Although fresh plants are preferable, preserved or dry herbarium specimens will answer very well.

#### *Sporophyte.*

1. Sketch an entire plant, carefully representing the four important regions of the sporophyte-sporangiophore, leaf blade, stem (short upright rhizome), and roots. Do the roots branch? Note the growing point at the summit of the rhizome from which new leaves are developed.

2. These four parts may be compared with the organs of a *Splachnum* sporophyte in a general way as follows:

- a. The sporangiophore with the capsule.
- b. The leaf with the hypophysis.
- c. The stem with the seta.
- d. The root system with the foot.

3. Study the venation of the leaf under low power. Draw a portion and describe. Study and draw the stomata.

4. Mount a part of the nearly mature sporangiophore. Draw under low power, showing the sporangia. Under high power draw some of the nonsexual spores.

LVI. *Botrychium simplex* Hitch. Little Grape-fern.

Class, Filices. Order, Ophioglossales. Family, Ophioglossaceæ.

This fern is to be found in moist woods and meadows.

1. Sketch the entire sporophyte and note the advance in complexity, over *Ophioglossum*, of the sporangiophore, leaf and roots.

LVII. *Botrychium lunaria* (L.) Sw. Moonwort.

The moonwort is found in the northern part of the United States and usually grows in fields.

1. Sketch the entire sporophyte, showing the sporangiophore, leaf, roots and rhizome. Describe how this plant differs from the preceding.

LVIII. *Botrychium matricariæfolium* A. Br. Matricary Grape-fern.

This fern grows in grassy woods and swamps.

1. Sketch the entire sporophyte and note the advance in complexity over the moonwort.

LIX. *Botrychium obliquum* Muhl. Oblique Grape-fern.

This grape-fern is widely distributed and may be collected in summer and autumn.

1. Sketch the entire sporophyte and note the advance in complexity over the preceding in the development of the sporangiphore, leaf and roots.

2. Study the venation of the leaf under low power. Is there any relation between the development of lobes and the venation?

3. Mount a branch of the sporangiphore and draw several sporangia under low power. Draw some of the nonsexual spores.

4. Ecological note. Notice the strong root-contraction and draw under dissecting microscope. How does the upright rhizome which continues to grow upward keep in the ground?

LX. *Botrychium virginianum* (L.) Sw. Virginia Grape-fern.

The Virginia grape-fern is common in rich woods and should be collected in the summer.

1. Sketch the entire sporophyte, showing especially the extreme complexity of the leaf. What relation is there between the ultimate segments of the leaflets and the venation?

2. Compare the last five plants in regard to the sporangiphore, the leaf-blade and the roots.

3. Cut cross sections of the rhizome, mount, and draw under low power, showing the wide cortex, the endodermis, the phloem, the cambium, the xylem (wood) with medullary rays, and the central pith.

4. Cut longitudinal sections of the fleshy root tips, mount the central sections, and draw a tip showing the apical cell.

5. The gametophytes of the grape-ferns are subterranean and difficult to find. They are destitute of chlorophyll and have the appearance of minute tubers. If fresh or preserved material of the gametophyte of the Virginia grape-fern is at hand, study and sketch under dissecting microscope or low power, showing the general contour of the body and the rhizoids.

6. Note. The advance from such forms as *Splachnum* and *Anthoceros* to *Ophioglossum* represents a vertical evolution, i. e. evolution upwards. The development indicated in passing through the series of forms from *Ophioglossum vulgatum* to *Botrychium virginianum* represents a horizontal evolution. There is a close relationship among these ferns. It must not be supposed, however, that the latter has necessarily been derived directly from the former, but only that the ancestors of *Botrychium* were at one time in a condition as simple as *Ophioglossum* is at the present time.

LXI. *Ferns. General study.*(a) *Adiantum capillus veneris* L. Venus-hair Fern.

Class, Filices. Order, Filicales. Family, Polypodiaceæ.

The Venus-hair fern grows in ravines and is widely distributed, but very rare in the North. It grows very readily in greenhouses, and is extensively cultivated. The gametophytes may be found at almost any time on pots in greenhouses. They may be raised in large quantities by sowing spores on any well-prepared, moist ground.

*Gametophyte.*

1. Mount a fresh, heart-shaped thallus in water and sketch it from the upper side under dissecting microscope.

2. Study the rhizoids under low power and draw a single one under high power. Are they unicellular or multicellular?

3. Under high power draw a single cell of the thallus, showing the chloroplasts.

4. Examine the lower side carefully under low power and note the numerous antheridia and archegonia. How are these organs distributed over the thallus? Under high power draw an antheridium and an archegonium (so much as can be seen of them above the surface of thallus). Compare the thallus of *Adiantum* with *Anthoceros* and *Marchantia*. Note especially the small comparative size.

5. Look for the large, spirally coiled spermatozoids moving in a ripe antheridium. Study free-swimming spermatozoids and draw. Describe the movement. The spermatozoids can usually be found on gametophytes of proper size and are often present in large numbers. Iodin will bring out the flagella.

6. If prepared slides are available, draw section of nearly mature antheridium, showing spermatozoids in various stages of development; also draw an archegonium, showing the neck, venter and oosphere.

*Sporophyte.*

7. Sketch and describe the compound leaf.

8. Mount and draw a single leaflet under dissecting microscope, showing the general outline and the free, dichotomous venation. How does the character of the venation explain the notched and cut margin? Can the origin of the leaflets be explained from the same standpoint? Note that the tips of some of the lobes are bent under so as to cover the sporangia.

9. Under high power study and draw the stomata. Are they on the upper or lower surface or on both? Draw a single cell, showing the chloroplasts.

10. If slides are at hand draw a section of a young sporophyte embryo, showing four definite regions (quadrants).

11. Pick out a young sporophyte from the under side of an old gametophyte, and draw under low power. Show the four regions, first leaf, root, bud and foot. Note that the young sporophyte is parasitic on the parent gametophyte, and that it acquires its independent life gradually.

(b) *Pteridium aquilinum* (L.) Kuhn. (Pteris). Eagle-fern.

Family, Polypodiaceæ.

The eagle-fern grows on hillsides, especially in sunny places. The rhizomes should be preserved in alcohol.

1. Cut cross sections of the rhizome, mount, and sketch under dissecting microscope, representing the following structures: the band of external sclerenchyma, the pith or ground tissue, internal sclerenchyma in two large, brown bands and in smaller patches, and the concentric fibro-vascular bundles—usually three large ones and a number of smaller ones. Note the two lateral ridges. How do you account for the dorsiventral condition of the rhizome?

2. Under high power, make a careful drawing of one of the smaller vascular bundles, showing the bundle sheath (usually brown), the phloem and the central xylem (wood).

3. Test for starch with iodine solution. Draw some of the ground tissue, showing the intercellular spaces, and starch in the cells.

4. Draw a patch of cells from the internal and from the external sclerenchyma, showing the thick cell walls.

5. Cut longitudinal sections of the rhizome, mount and draw, comparing the structures with those seen in the cross section.

6. Describe the mode of growth of the *Pteridium* rhizome. What advantages in the geophilous habit? Has this rhizome any advantage over the vertical rhizomes of *Ophioglossum* and *Botrychium*?

7. Carefully remove the leaves from the apex of a branch of the rhizome and cut cross sections of the growing point. Mount the sections, and in the first two or three look for the apical cell. Draw. Cut longitudinal sections of the apex of another branch, mount and draw the section, showing the apical cell. What is the shape of the apical cell?

8. Under dissecting microscope draw a leaflet of *Pteridium* from the lower side, showing the membranous false indusium formed of the reflexed margin of the leaflet.

(c) *Cyrtomium falcatum* J. Sm.

Family, Polypodiaceæ.

*Cyrtomium* grows readily in greenhouses and window gardens, and fresh sporangia may be obtained at almost any time of the year.

1. Examine a sterile leaf and a sporophyll. Draw one of the leaflets showing the circular sori on the under side.

2. Pick off some of the sori which have recently exposed the sporangia, examine and describe how the spores are scattered.

3. Mount an indusium and some opened and unopened sporangia. Draw the indusium under low power.

4. Draw a single sporangium under high power, showing the stalk, annulus, and lip cells. Contrast this sporangium with the one in *Botrychium*. Draw some of the nonsexual spores. Note shape, color, and surface.

5. Make a diagram in the notes showing the life cycle of a fern. See Fig. 11.



LXII. *Appendix to Ferns.*(a) *Camptosorus rhizophyllus* (L.) Link. Walking-fern.

Family, Polypodiaceæ.

The walking-fern is common on rocks, especially on limestone. Study fresh or herbarium specimens.

*Sporophyte.*

1. Sketch a plant with several leaves, some of which have rooted at the long acuminate tips, and show plantlets of various sizes. This is a simple and effective method of vegetative propagation. Leaves are usually highly specialized organs which have to a large extent lost the power of reproducing the individual. There are, however, many cases, like the present one, even in the higher plants, where the leaves retain the power of reproduction to a remarkable degree.

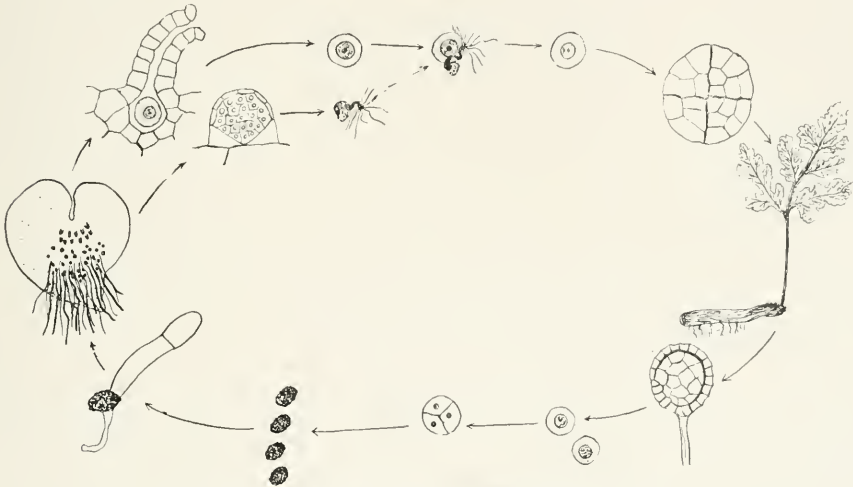


FIG. 11.—Diagram of life cycle of ordinary fern.

(b) *Filix bulbifera* (L.) Underw. (Cystopteris.) Bulb-bearing Bladder-fern.

Family, Polypodiaceæ.

This fern grows on moist rocks, especially limestone, and is easily cultivated in greenhouses, where it propagates itself extensively.

*Sporophyte.*

1. Sketch a leaf showing a number of fleshy brood-buds (bulblets). On which side of the leaf are they developed?

2. Under dissecting microscope draw a brood-bud which has just fallen off.

3. Draw a young fern plant which is developing from a brood-bud. Is this an efficient method of vegetative propagation? Why?

LXIII. *Lycopodium lucidulum* Michx. Shining Lycopod.

Class and order, Lycopodiales. Family, Lycopodiaceæ.

This lycopod grows in moist woods and on shady cliffs. Use fresh, alcoholic, or herbarium material.

*Sporophyte.*

1. Sketch the entire plant. Note the dichotomous branching, the alternating zones of sporophylls and sterile leaves, and the dichotomous roots.

2. Draw a branch, showing very carefully the tip and several zones of sporophylls below. Note that the formation of sporophylls does not stop the growth of the axis on which they are produced. Which are the larger, sporophylls or sterile leaves?

3. Draw a single sporophyll with sporangium under low power.

4. Under high power draw several nonsexual spores.

5. From alcoholic material cut cross sections of the stem, mount, and draw under low power. Note the epidermis with cuticle, the wide cortical layer, the vascular bundles of the leaf traces, the bundle sheath or endodermis, and the central cylindrical mass of vascular tissue. Inside of the endodermis are a number of more or less parallel strands of xylem and phloem. These structures will be more prominent after staining with iodine solution.

6. Cut radial longitudinal sections of the stem and compare in detail with the cross section.

7. Vegetative propagation. Notice the peculiar bulb-like brood-buds near the tips of some branches. Pick off one and draw under dissecting microscope.

LXIV. *Lycopodium obscurum* L. Ground-pine.

*Lycopodium obscurum* grows in moist woods, forming long slender rhizomes which creep under the surface of the ground or under leaf mould. From this rhizome upright, aerial branches develop.

*Sporophyte.*

1. Sketch an entire plant showing the rhizome and upright branch bearing a number of cones.

2. Draw a single cone under dissecting microscope. Note the spiral arrangement of the specialized sporophylls, and that by the development of a cone the further development of the axis is stopped. What is the probable reason for this?

3. Under low power draw a single sporophyll showing the sporangium. Note the advance in specialization of this sporophyll over that of the preceding plant.

4. Under high power draw some of the nonsexual spores; also some of the spore tetrads from younger sporangia.

5. Note.—This cone represents a primitive flower. Compare it with the zone of sporophylls in the preceding species.

LXV. (a) *Equisetum arvense* L. Field Horsetail.

Class and order, Equisetales. Family, Equisetaceæ.

The field horsetail is common along roadsides and railways, on river banks and steep slopes facing the north. The fertile branches come up in April and May, while the sterile ones begin to appear at about the same time, but do not reach their full development until later in the season. Spores may be collected

in large quantities and kept in a dry glass bottle. Rhizomes with fertile and sterile branches should be preserved in 70 per cent. alcohol. Good herbarium specimens may also be used.

*Sporophyte.*

1. Sketch a plant containing the rhizome, fertile shoot with cone, and young sterile shoot. Note the whorls of scale-like leaves at the nodes; also the lack of chlorophyll in the fertile shoot.

2. Sketch a mature sterile shoot.

3. Note and describe the division of labor in the stem of this plant—rhizome for a food storehouse and for vegetative propagation, fertile branch for the production of nonsexual spores, sterile branch with abundant chlorophyll for food manufacture. From whence is the material obtained which goes to form the fertile shoot?

4. Cut off some of the peltate sporophylls, mount and draw from the side under dissecting microscope. Show the stalk, the angular outer expansion and the sack-like sporangia hanging from the under side. How are the sporophylls arranged in the cone? Compare this cone and the sporophylls with those of *Lycopodium obscurum*.

5. Place a small flake of the dry spores on a slide without water or cover-glass, breath on them gently until the glass becomes moist, and examine immediately under low power. Note the spores with appendages coiled about them. In a few moments the spores will be in violent agitation, while the appendages uncoil. Breath gently on the slide while looking into the microscope. How many appendages on each spore? Draw. Describe in detail the hygroscopic properties of the appendages. Of what advantage to the plant is this peculiar arrangement?

6. Cut cross sections of a young fertile branch from alcoholic material. Mount, stain with iodine solution and draw under low power. Note the epidermis, the wide cortical layer with a circle of lysigenous cavities, the endodermis, the circle of vascular bundles, and the pith with a large central lysigenous cavity. The xylem (wood) of each vascular bundle is arranged somewhat in the form of a V, the apex of the V being occupied by a large air-cavity. The two limbs of the V end near the endodermis, and the phloem is situated between these two masses of xylem.

(b) *Equisetum hyemale* L. Scouring Rush.

This plant grows in wet places along the banks of rivers, creeks and lakes.

1. Examine the fresh or dry stems under low power. Notice the parallel grooves and ridges, with lines of tubercles and stomata. Draw and describe.

2. Break some of the dry stems and note their brittleness. Burn one of the stems in a hot flame, mount the outer part of the shell which remains, and examine under low power. Notice that the cell walls and stomata are still distinct. This is because the cell walls are impregnated with silica. Draw a flake showing the stomata.

## SUBKINGDOM, PTERIDOPHYTA. (HETEROSPOROUS.)

LXVI. *Marsilea quadrifolia* L. European Marsilea.

Class, Hydropterides. Order, Marsileales. Family, Marsileaceæ.

This water fern grows well in artificial ponds, in gardens and greenhouses. The western *Marsilea vestita* H. & G. found in wet places and shallow ditches on the great plains and prairies of the interior may also be used.

*Sporophyte.*

1. Sketch a branch of the creeping rhizome, showing the roots and the leaves with slender upright petioles.
2. Sketch a sporophyll with two sporocarps.
3. Carefully cut off part of the thick inner margin of some sporocarps and place them in a glass of water. In a day or two a gelatinous ring will be extruded on which are situated the sack-like sori in which microsporangia and megasporangia are contained. Draw.
4. Mount some of the microsporangia and megasporangia and draw each under low power. The megasporangium contains a single megaspore; the microsporangium a considerable number of microspores.
5. Under high power draw a single microspore and a megaspore, in correct proportion.

*Gametophyte.*

6. In the meantime the spores will begin to develop the gametophytes. These are very minute, and the spores in the water should be examined from time to time in order to get the proper stages. The male gametophyte develops entirely inside of the microspore wall and the female gametophyte merely protrudes the neck of the archegonium from one end of the spore. Draw a male gametophyte with a protrusion on the side of the spore wall for the escape of the spermatozooids and a female gametophyte with archegonium projecting from one end, showing a large number of spermatozooids in the gelatinous substance extending from the neck of the archegonium. Why does the microspore always give rise to a male plant, and the megaspore to a female?

7. If prepared slides are at hand, study and draw sections of mature male and female gametophytes. The male gametophyte corresponds to the pollen grain of seed plants, and the female gametophyte to the embryo-sac in the ovule. Both gametophytes of *Marsilea* must be compared with the hermaphrodite gametophyte of *Adiantum*. Note especially the great reduction in size; also that after this there will be no more hermaphrodite gametophytes, hence no possibility of self-fertilization.

8. In a week or so the female plants in the glass of water will have embryo sporophytes. Draw under low power and describe.

9. Ecological note.—Examine a plant at night, or place a flower-pot with a living plant in a dark chamber and note the manner in which the leaflets fold up. How long does it take the leaflets to unfold after being placed in sunlight?



LXVII. *Salvinia natans* (L.) Hoff. Salvinia.

Class, Hydropterides. Order, Salviniales. Family, Salviniaceæ.

This floating water fern grows readily in aquaria in greenhouses.

*Sporophyte.*

1. Draw an entire plant as it floats on the surface of the water, showing the horizontal stem, the leaves, and the peculiar root-like leaves hanging down from the underside.

2. Take out some of the plants and throw them into the water. Note how they nearly always turn right side up.

3. Place a leaf on the slide and examine without cover-glass under low power. Draw a part of the surface showing the peculiar hairs. What is their use?

4. Mount one of the dissected, root-like leaves and sketch under low power.

5. Ecological note.—Describe the various ways in which the sporophyte of *Salvinia* is adapted to its environment.

LXVIII. *Isoetes melanopoda* J. Gay. Black-based Quillwort.

Class and order, Isoetales. Family, Isoetaceæ.

This quillwort may be found in moist prairies and overflowed fields in the central states of the Mississippi valley. Fresh or herbarium specimens may be used, and stems preserved in 70 per cent. alcohol.

*Sporophyte.*

1. Sketch and describe the entire sporophyte, showing leaves, short stem, and roots.

2. Study prepared slides or cut cross sections of stems in alcohol and draw, showing the following structures: the two vertical furrows and two large lateral lobes, the outer cortex and extensive parenchymatous tissue in which the cells are arranged in radial rows, on the inner limits of this layer a zone of meristematic cells, inside of this a layer of clear cells (the phloem, "prismatic layer") and in the center a xylem-cylinder from which bundles pass outward to the leaves.

LXIX. *Sigillaria* Sp.

Order, Lepidophytales. Family, Sigillariaceæ.

Fossil impressions of the trunks of large, arboreous *Sigillarias* are common in the formations of the carboniferous period and may be seen in most museums.

1. Sketch the surface of part of a trunk of *Sigillaria*, showing the leaf scars and the longitudinal fluting.

2. Note.—The heterosporous pteridophytes of the present time are the remnants of a once great group of plants which formed a characteristic vegetation before and during the carboniferous period, which ended millions of years ago.

LXX. *Selaginella kraussiana* (Kunze) A. Br. Krauss' Selaginella.

Class and order, Selaginellales. Family, Selaginellaceæ.

This plant grows very luxuriantly in greenhouses and window gardens, if the soil is provided with proper moisture. Suitable material may be had at any time of the year.

*Sporophyte.*

1. Sketch an entire plant, showing branches, leaves, and roots. Note that the branches occur only in one plane and that the roots are dichotomous. Describe the character and arrangement of the leaves. How do you account for the arrangement? How does the plant accomplish vegetative propagation?

2. Draw a leaf under low power. Under high power draw a single cell, with chloroplast, from the lower surface. Draw also one of the stomata. Where are the stomata situated? Look for the ligule on the leaf.

3. Cut cross sections of a fresh stem or of stems preserved in alcohol, mount, and draw, representing the following structures: epidermis, cortical tissue in which may appear sections of bundles passing to the leaves, two or more large air spaces, and in the center of each space a vascular bundle. The bundle consists of a central strand of xylem (wood) surrounded by a band of phloem which is enclosed in a large-celled bundle sheath. Note the strands of cells passing through the air space from the cortex to the vascular bundle.

4. Draw one of the short bisporangiate cones (primitive flower) under dissecting microscope, showing microsporophylls above and one or more megasporophylls below.

5. Carefully pick off a microsporophyll and a megasporophyll each with its sporangium, mount and draw under low power. Note the greater specialization in the arrangement of the sporangia over that of Marsilea. Note the numerous microspores in the microsporangium. How many spores in the megasporangium?

6. Draw a microspore and a megaspore in exact proportion under low power. How do you explain this difference in size of the nonsexual spores? Determine how many times greater in volume the megaspore is than the microspore.

*Gametophyte.*

7. From prepared slides draw the male and female gametophytes of Selaginella, the archegonium, and antheridium, and the oosphere and spermatozoid.

8. Make a diagram in the notes showing the life cycle of Selaginella. See Fig. 12.

9. Note.—It will be remembered that in the lowest archegoniates the gametophyte is the important plant in the life cycle, and that the sporophyte is very small. Now in the highest forms the tables are turned and the sporophyte has become *the* plant. Between such plants as Isoetes and Selaginella on the one hand and the lowest living seed plants on the other, there is a considerable hiatus, nevertheless it is not difficult to trace the transformation which was necessary in passing from the condition of heterosporous pteridiophytes to the lowest gymnosperms.

## SERIES III. SPERMATOPHYTA. Sub-kingdom, GYMNOSPERMAE.

LXXI. *Cycas revoluta* L. Cycad.

Class and order, Cycadales. Family, Cycadaceæ.

This plant is usually grown in greenhouses and conservatories. Herbarium and museum material should also be at hand.

1. Examine a living plant and describe its general characters. Sketch the stem, showing the scale leaves and one foliage leaf.

2. Draw a megasporophyll (Carpel) from herbarium specimens, showing the megasporangia or ovules. Note the similarity of the carpel to the foliage leaves. The carpels are produced in a whorl like the foliage leaves, and the stem continues to grow through the whorl. Compare this condition with the ordinary ferns and with *Lycopodium lucidulum*.

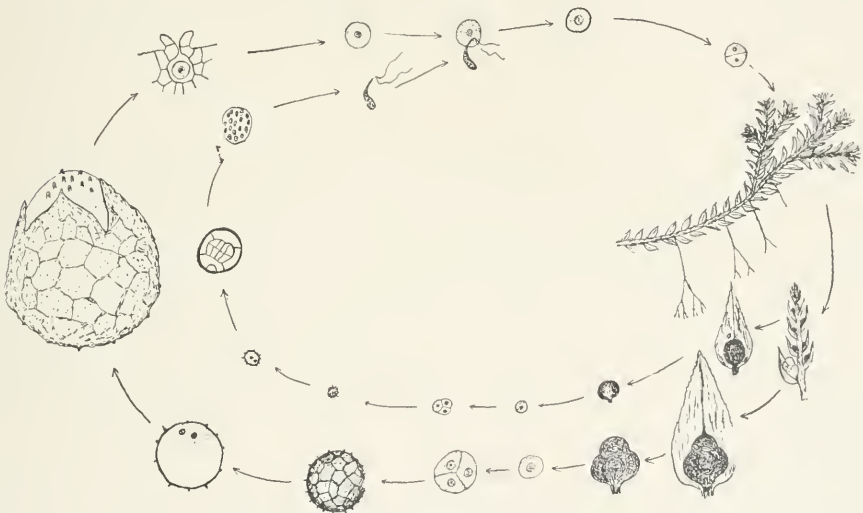


FIG. 12.—Diagram of life cycle of Selaginella.

3. Make a sketch of the large staminate (microsporangiate) cone. Draw a single microsporophyll (stamen), showing the numerous microsporangia (pollen-sacs) on the under side.

4. From alcoholic material draw a young ovule, properly dissected, showing the integument with micropyle, the inner wall of the ovule (megasporangial wall) with the pollen chamber, and the female gametophyte.

5. Draw half of a large female gametophyte from a mature seed, showing the little depression at the outer end and the dormant sporophyte embryo. The necks of the archegonia open into this depression (called the archegonial chamber) at the time of fertilization.

6. Mount male gametophytes (pollen-grains) and draw under high power.

7. If prepared slides are available study sections of pollen-grains showing the internal structure.

8. Note.—The fundamental difference between the heterosporous pterido-

phytes and the lower seed plants is that in the latter the microspores and megaspores are not shed, but develop the male and female gametophytes in the microsporangia and megasporangia respectively, while in the former the spores drop to the ground. The female gametophyte remains permanently enclosed in the megasporangium, but the male gametophytes are shed from the microsporangia and some fall into the micropyle of the ovule. This is known as pollination. In order that the spermatozoids may fertilize the oospheres in the archegonia a short pollen tube must grow through the tissue between the pollen chamber and the female gametophyte. It will be observed that the gametophytes are now entirely parasitic, the female in the ovule and the male at first in the pollen sac and, after pollination, in the wall of the ovule.

LXXII. *Ginkgo biloba* L. Maiden-hair Tree.

Class and order, Ginkgoales. Family, Ginkgoaceæ.

This beautiful tree, a native of China and Japan, is cultivated quite extensively in the United States. Museum and herbarium material may be used.

*Sporophyte.*

1. Sketch a leafy branch, showing the leaves developed in clusters on dwarf branches. Note that dwarf branches may give rise to ordinary branches.
2. Sketch a single leaf under dissecting microscope, showing the dichotomous venation. Compare the venation with that of the *Adiantum* leaf.
3. Sketch a stamen (microsporophyll) under low power. How many microsporangia or pollen sacs? Compare with stamen of *Cycas*.
4. Sketch a mature fleshy seed on its long stalk. Note the collar or cup around the base of the seed and the small undeveloped ovule. On some stalks two seeds develop. Remove the fleshy part of the integument and note the hard, inner layer.

*Gametophyte.*

5. Draw a male plant (pollen grain) under high power.
6. From alcoholic material study the mature female gametophyte (kernel of the seed). Sketch, and compare the size of the male and female gametophytes.
7. Carefully cut longitudinal sections from one side of the female gametophyte until the embryo sporophyte comes into view, and sketch the section under dissecting microscope, showing the embryo in position.

LXXIII. *Conifers. General Study.*

Class, Coniferæ. Order, Pinales.

The conifers called for below are cultivated quite extensively, and material for study can usually be obtained without difficulty.

(a) *Pinus*. Family, Pinaceæ.

Collect large branches of white pine (*Pinus strobus* L.), pitch pine (*P. rigida* Mill.), Austrian pine (*P. laricio* Poir.), and Scotch pine (*P. silvestris* L.). Also collect the dwarf branches with needle-leaves which have been self-pruned.



1. Study and sketch a branch of the Austrian pine, showing scale leaves, dwarf branches, and foliage leaves (needles). How old is the branch studied? What two ways of telling the age? Are the foliage leaves deciduous? How old are the dwarf branches before they are self-pruned? Where do the ordinary branches originate, and when?

2. Draw a dwarf branch, with scale leaves and foliage leaves, of the Austrian pine, Scotch pine, pitch pine, and white pine. Note the peculiarities of each dwarf branch.

3. Under low power, without cover-glass, draw part of the foliage leaf of the Austrian pine, showing the stomata. How are they arranged? Draw a scale leaf from the ordinary branch and one from the dwarf branch. Note the difference between the foliage leaves and the scale leaves.

4. Cut cross sections of a foliage leaf, mount and study under low power. Draw and note the following tissues: epidermis with sections of the stomata, heavy-walled hypodermal tissue, green mesophyll with a number of resin-ducts, and the light-colored central region with two vascular bundles.

(b) *Other Conifers.*

Collect branches of the following: Pinaceæ—the European larch (*Larix decidua* Mill.), Norway spruce (*Picea excelsa* (Lam.) Link.), Canadian hemlock (*Tsuga canadensis* (L.) Carr.); Juniperaceæ—arbor vitæ (*Thuja occidentalis* L.).

1. Sketch the larch branch, showing the large dwarf branches. Compare with Ginkgo and Pinus. Note that the foliage leaves are deciduous annually, and that the dwarf branches may develop into ordinary branches. Are the dwarf branches deciduous (self-pruned)?

2. Sketch a short branch of the Norway spruce and note a slight tendency to bilateral symmetry, and how the leaves are bent from the under side to obtain a proper light relation.

3. Sketch a branch of the Canadian hemlock with carpellate cone at the end. Note bilateral arrangement and the light relation of the leaves, especially the small ones on the upper side.

4. Sketch a small branch of the arbor vitæ. Note the flattened condition of the stem and the leaves. Note also that numerous branches of various sizes are self-pruned.

(c) *Structure of White Pine Stem.*

Preserve pieces of branches, five or six years old, in alcohol, and also obtain large, polished cross sections (about two inches thick) of a tree-trunk with bark.

1. With a strong, sharp razor, cut cross, tangential, and radial sections of stems in alcohol, mount, and stain with iodine; or study prepared slides.

2. Draw part of a cross section under low power, showing epidermis, cortex, with resin passages, phloem, cambium, xylem in a number of annual rings with medullary rays and resin passages, and central pith.

3. Radial section. Draw under low power, showing cortex, cambium, xylem (composed of tracheids), and pith. Note the medullary rays passing from the pith to the phloem.

4. Tangential section. Draw under low power, showing part of the xylem with tracheids and cross sections of the medullary rays.

5. Under high power draw part of a tracheid from radial section, showing the peculiar bordered pits.

6. Sketch part of a polished section of an old pine stem, showing bark, cambium, sap wood, heart wood, and pith. Notice the medullary rays. Notice also that each annual ring of wood is double—spring wood and fall wood. On which side is the spring wood? Describe the growth of a pine tree in height and thickness.

(d) *Sporophylls of Pinus laricio.*

Use fresh or alcoholic material.

1. Draw a staminate (microsporangiate) cone under dissecting microscope. Describe the arrangement of the stamens (microsporophylls).

2. Draw a stamen under low power, showing the outer (under) side with two microsporangia (pollen sacs). How different from the microsporophyll of *Selaginella* in structure and function?

3. Draw a young carpellate (megasporangiate) cone under dissecting microscope. Describe. Note that the parts are smaller at the lower end.

4. Draw a carpel (megasporophyll) from the lower side under low power, showing the bract (true leaf blade of the carpel) and the large ovuliferous scale. This may be an outward growth of the chalazal region of the ovules. Draw the carpel from the inner (upper) side, showing the two ovules (megasporeangia) and the ovuliferous scale. Compare the carpel with the megasporophyll of *Selaginella*.

5. Draw a mature carpellate cone. Note the spiral arrangement and that the carpels at the base are undeveloped and contain no seed. This is an example of rudimentary organs. Be careful to distinguish rudimentary organs (rudiments) from incipient organs (incepts) and from nascent organs.

6. Note.—The staminate and carpellate cones of the pine represent primitive flowers. Are these flowers monosporangiate (one kind of spores in the flower) or bisporangiate (both kinds of spores in the same flower)? Compare with the cone in *Selaginella*. Is the pine tree (sporophyte) monœcious or diœcious?

(e) *Carpellate Cone of Larix decidua.*

Collect carpellate cones of the usual type and some which have the tip continued as a leafy branch. Preserve in alcohol.

1. Sketch a normal cone in which terminal growth has been completely checked.

2. Sketch a cone on which a leafy branch has developed at the outer end. Note the gradual transition from carpels to ordinary foliage leaves. Sketch a number under dissecting microscope, showing this transition. This continued growth or prolongation of the floral axis of the larch cone is a good example of reversion to a more primitive condition or atavism. Compare with the ordinary ferns, *Lycopodium lucidulum*, and *Cycas*.

3. Observe fresh or dried, young cones and note the presence of a special color. How do you account for the color in this cone?

(f) *Gametophytes and seed.*

The gametophytes of *Pinus laricio* may be studied from staminate and carpellate cones preserved in alcohol. The seeds may be kept in a dry condition.

1. Draw a male gametophyte (pollen grain) under high power. Note the two wings. These represent an adaptation for anemophilous pollination.

2. Remove a female gametophyte from a young seed and draw under dissecting microscope. Note the difference in size between the male and female gametophytes. Compare the two gametophytes with those of *Marsilea* and *Selaginella*.

3. From the female gametophyte of a mature seed carefully cut out the embryo sporophyte, which is now in a dormant condition. Sketch under dissecting microscope, showing the radicle and cotyledons. Pick off the cotyledons from one side and sketch the plumule. How many cotyledons?

4. If prepared slides are at hand draw a section of a stamen, showing the one-celled microspores.

5. Draw a section of a male gametophyte, showing the large tube cell and nucleus, the generative cell and the two disorganized vegetative cells lying like two thin plates against the wall of the grain back of the generative cell.

6. Draw a section of a young ovule, showing the functional megaspore.

7. Draw a pollen grain which has formed a short pollen tube growing down into the nucellus (tip of the megasporangium). Note the tube nucleus in the tube and in the body of the grain the spermatogenous cell, the stalk cell and the remains of the two evanescent vegetative cells. The spermatogenous cell divides later into two sperm cells which do not have flagella or cilia. From the same section draw the spherical embryonic female gametophyte.

8. Draw a female gametophyte showing archegonia with oospheres.

9. Draw an archegonium in which the nucleus of the oosphere has divided into four nuclei.

10. Draw the upper part of a female gametophyte, showing remains of archegonia with an elongated cavity below them in which appear a number of embryos in various stages of development. Only one of these embryos survives, probably the one which has a slight advantage in size, vigor, and food supply. Note the struggle for existence which must go on among these embryos.

11. Sketch a mature seed, showing the wing. Let a winged seed drop to the floor from a height of six or seven feet and note how it falls. Describe the adaptation this seed has for dissemination. Note also the readiness with which the seed is separated from the wing. Of what use is this adaptation?

(g) *Seedlings and Primitive Leaf Arrangement.*

Plant seeds of *Pinus* and *Thuja occidentalis* and use fresh plantlets or preserve in alcohol. Also obtain branches of the common juniper (*Juniperus communis* L. Family, *Juniperaceæ*) and cultivated varieties of *Thuja* known as *retinispora* forms. In these *retinispora* or juvenile forms, branches often change

suddenly from the form with spreading leaves to the flattened condition, and the flattened branches again revert to the form with spreading leaves.

1. Sketch a pine seedling which has sprouted, showing the seed coat still covering the cotyledons. Sketch a seedling with cotyledons expanded. Describe the important changes which take place in the embryo during the process of sprouting.

2. Sketch a branch of *Juniperus communis* and note that all of the leaves are of the spreading type.

3. Study and sketch the seedlings of *Thuja occidentalis* and note that at first the leaves are of the spreading type, much like those of *Juniperus*, and that later the branches have the flattened form characteristic of the adult plant.

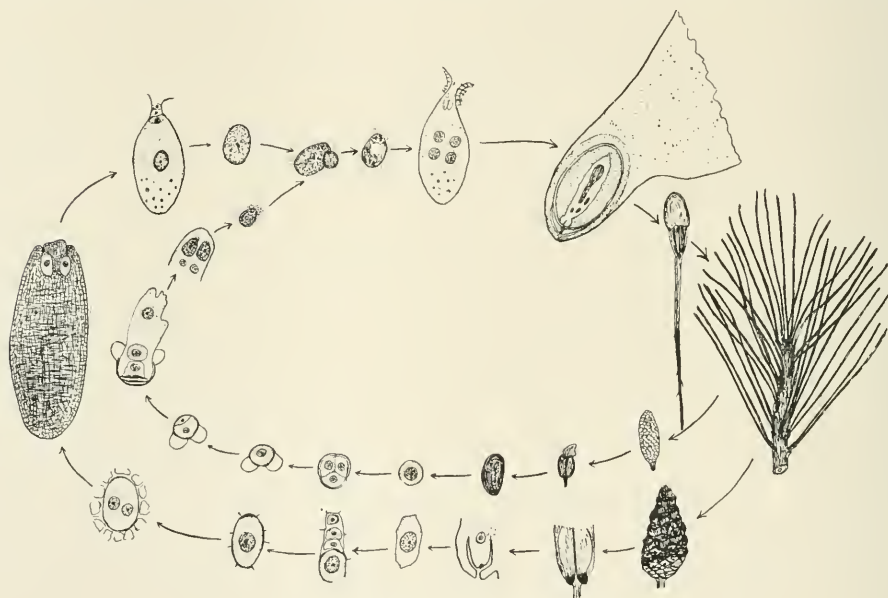


FIG. 13.—Diagram of life cycle of pine.

Apply the recapitulation theory as given in connection with the moss protonema. From this it would appear that the ancestors of *Thuja* had the leaves arranged like those of the common juniper.

4. Study and draw a small branch of *Thuja* (retinispora form) in which the upper part of a flattened branch has changed back to the juvenile form. In such cases there is a double reversion. In other words, the branch takes on first one form and then another successively.

5. Make a diagram showing the life cycle of a pine. See Fig. 13.

6. Note on the development of the carpellate pine cone.

The young carpellate cones of *Pinus laricio* begin to develop in the bud during summer or fall, and in the following spring the carpels have young ovules with a distinct integument. Later the ovules are pollinated and the megaspore is developed. In the following autumn (October) the megaspore has germinated and the female gametophyte is developing as a hollow spherical body composed



of free, naked cells. It passes the winter in this condition, and by the following June the archegonia with eggs are ready for fertilization and the pollen tubes have grown down through the nucellus. About the last week in June or the first in July fertilization occurs, and the embryo is matured and in the resting condition in the following autumn. The seed is usually shed late in the winter or in the early spring of the following year. The whole history thus covers nearly three full years.

LXXIV. *Taxus canadensis* Marsh. American Yew.

Class, Coniferæ. Order, Taxales. Family, Taxaceæ.

The yew is a low shrub growing on moist banks and hills, especially in the shade of large conifers. It is common northward. Herbarium and alcoholic material may be used if fresh branches are not available.

1. Sketch a branch, showing arrangement of leaves. Describe.
2. Under dissecting microscope draw a staminate cone. How are the stamens arranged?
3. Draw a single stamen under low power and note the peltate form. How many microsporangia? Compare the shape of this stamen with the sporophyll of *Equisetum*.
4. Under dissecting microscope draw a small fertile branch with a young ovule at the tip.
5. Cut longitudinal sections of the branch with ovule, mount, and draw under low power, showing the megasporangium in the center surrounded by the long inner integument and a short outer undeveloped aril, with scale-leaves on the stem below.
6. Draw a ripe seed with the thick, fleshy, red aril.

LXXV. *Higher Gymnosperms.*

Class and order, Gnetales.

Study herbarium specimens.

1. Make a sketch of a small plant of *Ephedra nevadensis* Wats. (Family, Ephedraceæ.) Note the slender green stems and the dry scale-leaves. In what ways is this plant adapted to a xerophytic environment?
2. Make a sketch of a branch of *Gnetum gnemon* L. (Family, Gnetaceæ.) Note the large, brood leaves. This is a tropical tree cultivated in India and surrounding regions.

JOHN H. SCHAFFNER.

Ohio State University.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

**Parkhurst, H. E.** Trees, Shrubs, and Vines of the Northeastern United States. 8vo. pp. 451. Illustrated. Charles Scribner's Sons. New York, 1903. Price, \$1.50.

This book presupposes no previous knowledge of botany, but is intended for the nature lover who wishes to become acquainted with the trees,

shrubs, and vines without the labor of a preliminary training in botanical science. Even in the descriptions, very few technical terms are used, and these are fully explained and illustrated. While the book is addressed to the non-botanical public, the keys cannot fail to be useful to the botanist as well, for in scientific works the keys are based so largely upon the details of floral structures that they are of little value except during the short flowering season. The keys of this book are based upon the leaf and other features of growth, and consequently are useful for fully half the year. The figures of leaves are very numerous and are very accurately drawn.

Considerable space is devoted to the trees, shrubs, and vines of Central Park in New York. This feature, however, is not one of mere local interest, for a survey of the list of forms in this park shows that it includes practically all of the plants which are likely to be met in any part of the northeastern United States.

The book can be recommended heartily to nature lovers, whether they live in the city or in the country.

C. J. C.

**Tischler, G.** Ueber Embryosack-Obliteration bei Bastardpflanzen. Beihefte zum Botanischen Centralblatt, 15: 408-420, pl. 5, 1903.

While it is well known that the pollen of sterile hybrids is often imperfect, comparatively little is known about the behavior of this embryosac in these

forms. The present paper deals with the embryo sac of *Ribes Gordonianum* Lem., a hybrid between *R. aureum* Pursh and *R. sanguineum* Pursh, and with the embryo sac of *Syringa chinensis*, a hybrid between *S. vulgaris* and *S. persica*. Both parents of *Ribes Gordonianum* have normal embryosacs with conspicuous nutritive tissue in the chalazal region of the ovule. In the hybrid, this nutritive tissue is lacking and the development of the embryosac is usually checked long before it reaches the fertilization period, the megaspores often failing to germinate at all.

In the parents of *Syringa chinensis* the nutritive tissue is in the form of a jacket surrounding the embryosac, which, in both cases, is normally developed. In the hybrid the nutritive jacket is more highly developed than in the parents, but the embryosac becomes disorganized quite early, so that the stage at which fertilization might occur is seldom or never reached.

References are given to the few instances previously described of irregularities and imperfections in the development of the ovules and embryosacs of sterile hybrids.

C. J. C.

# CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE MOODY.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole Moody, 125 Belvedere Street, San Francisco, Cal.

**Beard, J.** Embryology of Tumors. *Anat. Anz.* 23: 18-19, 486-494, 1903. The author is applying his researches on the distribution of germ-cells to the

origin of these pathological growths. Tumors are shown to be referable to abnormal attempts at development on the part of one or more vagrant or aberrant primary germ-cells and also to the bizzare pathological manifestations by such of some greater or less portion of the life cycle of normal development. The author's researches have revealed the fundamentally impossible nature of three tenets of modern embryology: somatic origin of germ-cells, direct development, epigenesis. Continuity of the germ-cell from generation to generation is now an accepted fact. As these cells do not arise at the first division of the fertilized egg but at a later definite period, there always are some cleavage products concerned neither in the formation of the embryo nor of germ-cells. The products give rise to an asexual foundation, larva, phorozoön upon which the germ-cells and with these an embryo take origin. In human development this is the chorion. The formation of an embryo is only a part of the life cycle. The set of germ-cells is produced, each being a potential embryo. If two primary germ-cells undergo normal development, identical twins result. If these should develop together or at different times with abnormalities on the part of one, a rudimentary embryo, an embryoma or a tumor may result. The primary germ-cells are constant in number for a given species, 8 in frog, 32 in lamprey, 128 in dog-fish. One deducted from the number to form an embryo leaves the remainder to wander into the embryonic body to form the reproductive products. Any of these vagrant cells may fail to reach the normal position and thus by being included in other organs and parts "infect" the whole body. Tumors form a series of parasites comparable to the eulima series of snails parasitic on star-fish and other echinoderms. A tumor is a more or less reduced incompletely differentiated sterile metazoan organism. It is started from a vagrant germ-cell, and owing to abnormal conditions develops part of its possibilities. "Hence they are not *part* of the organism in which they occur, but reduced *sisters* or *brothers*."

A. C. M.

**Bensley, R. R.** Concerning the Glands of Brunner. *Anat. Anz.* 23: 497-507, 1903. The author studied these glands in twenty species of mammals, and succeeded in getting a modified muchematin stain that differentiated the double nature of the glands in all cases. By increasing the given strength of the stain solution with altering the proportion of the solid constituents, sections fastened to the slide and in celloidin were well stained. The solution is prepared as

follows: hematin 1 g., aluminium chloride .5 g. are rubbed together in a mortar and dissolved in 100 ccm. of alcohol diluted in tap water to 70 per cent. This solution stands a week, deepening in color and increasing in staining capacity. Then the stain is tested on a section. If the resulting stain is diffuse 10 per cent. solution of nitric acid is added drop by drop, the stain being tested between each drop added. When the mucin cells stain a deep blue rapidly, cytoplasm and other tissues being unstained, the reaction is right. Slightly over-acid stains give good results, except that the nuclei have a meta-chromatic red stain. The solution is used as follows: sections cut in paraffin and fastened to the slide by the water method are treated with benzole followed by absolute alcohol. With the slide under the microscope a drop of stain is put on and left until with a low power the secretion of the cells is deep blue. Wash rapidly in 70 per cent. alcohol, dehydrate, clear in xylol and mount in balsam. Prolonging the staining and non-renewal of the solution from time to time fail to give permanent preparations; no water should be put on the stained sections. If water is needed the stain may be made resistant to it by placing the slide for a few moments in a saturated solution of copper acetate in alcohol, or in a solution of sodium hydroxide, both of which form a compound with hematoxylin.

Mucicarmine gave successful results with the glands of Brunner if a strong stock solution of Mayer, freshly prepared, is used. After 24 to 48 hours the solution cannot be filtered and refuses to stain. Both these stains will bring out secretions from the following: mucous cells of salivary, buccal, lingual, palatine, tracheal and esophageal glands, gastric epithelial cells, cells of cardiac glands of stomach, pyloric gland cells; the neck chief cells of the gastric glands, goblet cells and glands of Brunner of the rabbit. They do not stain secretions of the following: demilune cells of salivary glands; serous salivary glands; from pure or mixed glands; serous portion of palatine and tracheal glands; glands of v. Elner in the tongue; the chief cells of the body of the fundus glands of the stomach; parietal cells of fundus gland; pancreatic cells.

A. C. M.

**Marpmann, G.** New Imbedding Medium. *Zeitschr. angew. Mikr.* 9: 14-16, 1903. (Rev. J. R. M. S. 4, 558, 1903.)

The author recommends celluloid dissolved in acetone as a cheap, effective substitute for celloidin. Celluloid chips are placed in a wide necked bottle and covered with acetone (10 x bulk). The tightly covered bottle should be shaken at intervals and then allowed to stand till all the celluloid is dissolved. The clear liquid is then poured off. Two solutions are required, one thin, one thick, syrupy in consistency. Perfectly dehydrated material is placed in the thin solution for several days and then some of the thick is added. The medium is thickened by slow evaporation under a bell jar. Blocks should be free from cracks or holes, and can be kept in 80 per cent. alcohol; sections may be mounted as they are or the celluloid dissolved out by means of acetone.

A. C. M.



## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

**Streeter, G. L.** Ueber die Verwendung der Paraffineinbettung bei Markscheidenfärbung. Arch. f. mik. Anat. **62**: 734-740, 1903.

by the Weigert method. He takes advantage of the fact that hæmatoxylin forms a compound insoluble in xylol or paraffin when brought in contact with chromordanted myelin. Fresh material is mordanted at room temperature for 4 to 8 days in mixture of 5 per cent. potassium bichromate and 2 per cent. fluorchrom. The slower acting (2 to 3 months) 5 per cent. potassium bichromate, or Muller's fluid may be used to even greater advantage by reason of more uniform penetration. Formol and copper acetate are to be avoided because of interference with subsequent staining. The fixing agent is washed out very thoroughly in 80 per cent. alcohol changed frequently for 2 to 3 weeks or in running water. Long exposure to alcohol is no detriment. The preparations are then stained *in toto* in Weigert's lithium carbonate-hæmatoxylin, the stain being renewed at 24 and 72 hours. After washing for 48 hours in 70 per cent. alcohol the objects are embedded in paraffin by either the chloroform or xylol method, the usual procedure being followed. For small animals sections 10 to 15  $\mu$  in thickness are to be preferred. To prevent possible loss of sections from the slide a thin coat of celloidin is spread over them after dipping the slide in alcohol-ether. Sections were differentiated in Weigert's ferricyanide of potash (2½ per cent.)—borax (2 per cent.) solution diluted ten times, or with better results by Pal's method, washed for 48 hours in running water and by the usual procedure—alcohols, carbo-xylol, and xylol—carried into balsam. The results are most satisfactory in economy of time, perfection of series and completeness of the staining. Experiments are in progress in quest of a stronger stain with greater capacity of penetration.

C. A. K.

**Cohn, F.** Zur Histologie und Histogenese des Corpus luteum und des interstitiellen Ovarialgewebes. Arch. f. mik. Anat. **62**: 745-772, Taf. 31 und 8 figs. im Text, 1903.

in thickness were stained in hæmatoxylin-eosin, in Mallory's phospho-tungstic acid-hæmatoxylin, in Heidenhain's iron-hæmatoxylin, or in Rabinovic's hæmatoxylin devised for nerve staining. This last stain was especially favorable for the demonstration of the structure of the protoplasm. Mallory's connective tissue stain was used to demonstrate the thecal connective tissue in the corpus luteum.

C. A. K.

The author suggests a procedure which obviates the necessity of preparing sections by the celloidin method in staining the myelin sheath of nerve fibres

Rabbit ovaries taken from 20½ hours to 15 days *post coitum* were fixed in Tellyesniczky's acetic-bichromate or in Zenker's fluid. Sections 4 to 10  $\mu$

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory,  
University of Michigan, Ann Arbor, Mich.

Macallum, A. B. On the Inorganic Composition of the Medusæ, *Aurelia flavidula* and *Cyanea arctica*. Jour. Physiol. 29: 214-241, 1903.

As a necessary preliminary to some physiological experiments on the effect of chemicals on medusæ, Professor Macallum has determined the inorganic composition of two common medusæ, paying especial attention to the difference in composition of the organisms and of the sea water in which they live. He finds that the degree of salinity of *Aurelia flavidula* and *Cyanea arctica*, as indicated by the amount of total halogen, may be and usually is different from that determined in the sea water from which they are taken. The two forms show a different degree of salinity though taken at the same time from the same water. The salinity of the sea water may vary widely without affecting, except to a small extent, the salinity of the organisms. The salts once deposited in the jelly of the organisms, so long as these are living and the medium is sea water, do not appear to be affected appreciably by the osmotic pressure, and the salinity, as indicated by the total halogen, would appear to be due to additions merely from the salinities of the various sea waters in which the animal has lived. In these medusæ the sodium is slightly less and the potassium considerably more than in the sea water. The amounts of potassium are in the proportions which exist between the amounts of proteid nitrogen found in the organisms. The magnesia is less (10 per cent.) than in sea water and the sulphuric acid ( $\text{SO}_3$ ) is also deficient (to 32-36 per cent.). The iron is more, and the iodine less, abundant than in sea water. These results indicate that the cells lining the gastrovascular channels, and perhaps also to some extent those covering the organisms, exert a selective action in absorbing the salts of sea water and that the selective action varies, being more vigorous in the case of some constituents than in others. This selective action is referable to the cells considered as living units.

R. P.

Gaylord, H. R., and Wheeler, D. E. On the Destruction of Bacteria in Vaccine Pulp with Potassium Cyanide. Amer. Med. 6: 349-352, 1903.

This paper is of interest to physiologists as an example of the turning to practical use of results obtained in the domain of comparative physiology.

Some time ago Loeb and Lewis found that by placing sea urchin eggs in weak solutions of KCN in sea water the eggs could be kept alive much longer than if they were placed in sea water alone. Later Gorham and Tower pointed out that this result was due to the fact that the KCN solution used killed the bacteria in the sea water without affecting the protoplasm of the eggs, and consequently provided a practically sterile solution for the eggs. Gaylord and Wheeler were led by these results to experiment with vaccine virus with a view of determining

whether there might be found a strength of potassium cyanide which would destroy the bacteria in vaccine without injuring the essential organism. Their efforts were entirely successful. They found that a solution of  $n/200$  KCN exerts a complete bactericidal action upon the vaccine without in any way injuring the specific organism. Successful vaccinations were made on children with pulp which had been freed from bacteria by the KCN. The authors suggest that this result gives inferential evidence, at least, that the "organism of vaccine partakes of the nature of animal protoplasm and is a protozoon." R. P.

**Snyder, H.** The Chemistry of Plant and Animal Life. Easton, Pa. (Chemical Publishing Co.), 1903.

This work is stated by the author to be the outgrowth of his teaching of chemistry in the School of Agriculture of

the University of Minnesota, and it is essentially a combination of lecture work and laboratory directions for an elementary course in certain phases of agricultural chemistry. The first twenty-one chapters (154 pages) are given up to a discussion of elementary general chemistry. The greater part of the remainder of the book deals with the chemistry of plants chiefly from the standpoint of the agriculturist. Only a few chapters are devoted to animals, these dealing with the composition of the animal body, and animal foods. The chief value of the book to the general physiologist is found in the fact that it presents in a handy form for reference, a digest of much of the work which has been done by the chemists of the various experiment stations. R. P.

**Fischer, E.** Experimentelle Untersuchungen über die Vererbung erworbener Eigenschaften. Allg. Zeitschr. f. Entomol. Bd. 6, 1901.

——— Weitere Untersuchungen über die Vererbung erworbener Eigenschaften. Ibid. Bd. 7, 1902.

Rev. in Zeitschr. f. Allgm. Physiol. Bd. 3. Ref. pp. 82-88, 1903.

The principal result of Fischer's work on the inheritance of acquired characters appears to be very well established, and it is one of fundamental significance to biological thought. In the course of his study of color and form

changes artificially induced in butterflies by temperature differences, Fischer has found that such "acquired characters" may be inherited. A particular favorable form for such work is *Arctia caja* L. By the action of cold ( $-8^{\circ}$  C.) on the pupæ aberrant adults are produced differing from the normal individuals not only in color, but in color pattern and general form. When such aberrant individuals are bred under normal conditions nearly 10 per cent. of the progeny again show the aberration in the adults. These results show that it is possible for temperature influence to produce progressive changes in the species in a state of nature. R. P.

**Rádl, Em.** Untersuchungen über den Phototropismus der Tiere. Leipzig (Engelmann), pp. VIII and 188, 1903. (4Mk.)

In this work the author gives a critical summary of practically all of the work which has been done on the reactions

of animals to photic stimuli, together with the results of his own investigations in this field. The extent of the field covered and the mass of detailed facts which the work comprises make it impossible to give a brief summary of the results. Rádl's own work on the light reactions of organisms (some of which has already been reviewed in this department) has been carried on from a distinctly original point of view, and he has brought out a number of facts, which are very interesting at least. His general standpoint is that the phototropic orientation of an organism and the optical orientation of man are "ganz analoge Erscheinungen." R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt,  
Harvard University Medical School, Boston, Mass.

**Reed, Dorothy.** A case of acute lymphatic leukæmia without enlargement of the lymph glands. *Am. Jour. Med. Sci.* 1902, cxxiv, p. 653.

A careful histological study by Reed supports the view of Walz and Pappenheim that in every case of leukæmia the primary seat of the disease is in

the bone marrow. The old theory that lymphatic leukæmia is essentially a disease of the lymph nodes, is still supported by Ehrlich. He bases his claim on the assertion that no lymphocytes occur normally in the bone marrow. This statement the writer calls into question, as in the bone marrow cells are found identical morphologically with the lymphocyte. There is no proof that the lymphocytes of the blood come from the lymph glands alone, and although it cannot be stated positively that they arise in the bone marrow it is known that all other colorless cells of the adult blood originate in the bone marrow from lymphoid cells and that cells identical morphologically with the lymphocytes of the blood are found there.

The case upon which this study was based ran a rapid course. Hæmorrhages, anæmia and progressive weakness were the chief clinical features. A thorough study of the blood was not made during life. At autopsy neither spleen nor lymph nodes were enlarged. The bone marrow of the femur was homogeneous, deep red, and very soft. Microscopically the bone marrow consisted chiefly of lymphoid elements. The predominating cell was smaller than the red blood corpuscle and possessed a densely staining homogeneous round nucleus, and a small amount of clear protoplasm. There was another type of lymphoid cell varying in size which possessed a nucleus relatively large in respect to its protoplasm. The nucleus always showed a definite chromatin network. The cell was identical in structure with the lymphocytes of the blood and of the lymph nodes. There was no indication of hyperplasia in either the spleen or lymph nodes.

This case is not unique. Six similar cases have been recorded within the last few years, and authorities are now nearly agreed that in lymphatic leukæmia the blood changes do not occur until the bone marrow is involved. No case of leukæmia has come to autopsy in which the bone marrow was normal.

If leukæmia is always a disease of the bone marrow it is manifestly incorrect to restrict the term Myelogenous to one form of the disease. The writer suggests a new clinical classification based upon the blood picture. Three forms of leukæmia are recognized, all due to myelogenous changes,—the myelocytic, the lymphoid, and the mixed cell varieties. Leukæmia resembles an infectious disease. Possibly in leukæmia a positive chemiotaxis exerted by some poison in the body draws out certain cells from the bone marrow into the blood.

J. H. P.



## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn,  
Wesleyan University, Middletown, Conn.

**Gage and Phelps.** On the classification and identification of Bacteria with a description of the card system in use at the Lawrence Experiment Station for Records of Species. Proc. Am. Pub. Health Ass., 1902.

These authors have made an actual contribution to the vexed problem of systematic bacteriology. Guided by the numerical system of records sug-

gested first by Kendall, they have modified this system so as to record all characters which are in use for the distinction of species. This has been accomplished by a grouping of characters, impossible to describe in an abstract, but simple in application. They have further devised a record card for specific descriptions upon which a complete description of any bacterium can be entered, everything being recorded by numbers. The distinctive numbers are placed in a single line on the top of the card, making comparisons very easy. By the system of card cataloguing these cards can be filled in such a way as to bring together species with similar numbering, and hence close relations. The plan is calculated to simplify the difficult task of keeping bacteriological records, and will aid much in bringing order out of the chaos of systematic bacteriology. No bacteriologist who is concerned with water bacteria, or any other branch of the subject that involves the study of numerous species, can afford to miss the careful consideration of this simple means of recording results.

**Kendall.** A Proposed Classification and Method of Graphical Tabulation of the Characters of Bacteria. Proc. Am. Pub. Health Association, 1902.

A second article, which is, in a way, an extension of the above is by Kendall, published in the same journal. This

is a further attempt to reduce to a numerical form a method of recording the properties of bacteria. The author uses the nomenclature given by Chester and assigns arbitrarily certain numbers to various terms used in the descriptions. The result is that by the use of these numbers a very condensed description of species of bacteria is possible. This article together with that of Gage and Phelps will certainly form a basis of bacteriological records and will be of inestimable value in systematizing bacteriological data.

**Rogozinski.** Ueber die physiologische Resorption von Bakterien aus dem Darm. Hyg. Rund., p. 926, 1903.

A study of the vexed question whether micro-organisms are capable of migrating from the alimentary canal is the

subject of this research. The method adopted for study was to narcotize the animal, and opening the body before the animal is dead, to remove for study portions of the living organs. Small quantities of such materials, taken with the greatest precautions to prevent contamination, are inoculated into boullion, and allowed to grow, after which plates are made from the boullion culture. Various tests convinced the author that the method is satisfactory, and that his results are not due to contamination. He finds in general that the mesenteric glands practically always contain bacteria, whereas the chyle, the liver, and the spleen are free from them.

## GENERAL LABORATORY TECHNIQUE.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory,  
University of Michigan, Ann Arbor, Mich.

### A Method of Obtaining Uniplanar Sections with the Ordinary Rocking Microtome.

One of the chief objections to that otherwise very satisfactory piece of laboratory apparatus, the Cambridge Rocking Microtome of the old pattern, has always been that the sections cut were not uniplanar, but were instead segments of a cylinder. While this defect is not serious when the area of the face of the block is small, it becomes an important matter when the area is large. W. S. Handley (*Jour. Anat. Physiol.*, Vol. XXXVI, pp. 290-292, 1903) has described a method of obviating this defect which will be found useful by users of the type of microtome mentioned. His method essentially consists in embedding the slice of tissue to be cut on a cylindrical surface, corresponding to the curve described by the rocking arm of the microtome. "A squared block of paraffin, sufficiently large to contain the piece of tissue to be cut, is fixed on the rocking arm, with its center truly in the axis of the arm, with its upper edge horizontal, and with its free surface forming a plane at right angles to the axis of the arm. These points can be judged with sufficient accuracy by the eye. This pattern block is now cut in the ordinary way until its cut surface has acquired the convex cylindrical form, and complete sections of it are being cut by the razor. It is now dismantled, covered with thin tinfoil, and surrounded by a projecting rim of stamp-paper. A mould with a convex cylindrical floor is thus produced, and into this plaster of paris is poured as it stands on a level surface. When the plaster has set, it forms a square or oblong block with one concave cylindrical surface. Tinfoil is swagged down upon this surface, and the edges of the block surrounded by a rim of stamp-paper. The embedding mould is now ready. The slice of tissue is very thin, say not more than one-eighth inch thick, for two reasons. First, since the sections are uniplanar, *very much thinner pieces may be used*, which is one great advantage of this method. Secondly, unless the piece of tissue is thin, it does not adapt itself properly to the concave floor of the embedding mould. During the process of embedding it is necessary to hold down the slice of tissue with hot needles until the paraffin has set sufficiently to hold it in its curved position on the floor of the mould. While the paraffin is cooling, the mould must of course stand on a horizontal surface. The paraffin block thus obtained must be detached from the mould and fixed truly on the rocking-arm, in a position exactly corresponding to that of the pattern-block from which the mould was made. The razor will at once commence to cut complete sections of the curved surface of the block."

Handley states that by this method he has been able to obtain uniplanar sections from blocks  $1\frac{1}{4}$  inches in diameter without difficulty. R. P.

### Ink for Glass.

The *Pharmaceutical Era* (September 24, 1903) gives the following method of making ink for writing on glass: Dissolve 20 parts resin in 150 parts of alcohol and add to this solution, drop by drop, and under continuous stirring, a solution of 35 parts borax in 250 parts water. Finally dissolve 1 part methylene blue in the mixture. R. P.

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## The Microscope and Expert Testimony.

The microscope is indispensable if the facts regarding certain classes of questioned documents are to be known and shown. Such an instrument, however, is still somewhat of a novelty in a court of law, because for so long a time the jurymen was not supposed to be able to see things for himself, but simply listened to reports of observations made by others and acted thereon. If the observers disagreed he was helpless, as he still is regarding things disputed which he cannot see and understand; but now in many cases an intelligent jurymen, judge or referee cannot be misled either by a lawyer who is opposed to the fact or by witnesses who bear false witness.

Questions arise in many ways in connection with a study of the various phases of forgery which the microscope alone can answer, and in many cases its reply is conclusive. The instrument is useful in this as in other fields because it enables the observer to see clearly what otherwise is invisible or can be seen only indistinctly; and facts and phenomena are not less significant, if they actually exist, simply because the unaided eye cannot see them. There is a whole microscopic world, as real as the world of common things, and it is into this realm that the microscope leads.

The average unaided or naked eye can distinguish separate objects or lines up to only about two hundred to the inch, more than this appearing as a solid shade or tint. This being the fact it is easy to understand, for example, how far beyond human sight are the individual red blood corpuscles when we know that something more than three thousand of them laid side by side extend only an inch. They are large compared with many microscopic objects, but are as far away from unaided vision as the beautiful rings of Saturn were before the invention of the telescope. Many facts of importance in legal inquiries are also absolutely invisible without the microscope, and without its assistance we may indeed have eyes and see not.

In law, inanimate things may become instruments of evidence and speak for themselves, needing no other testimony, and justice is always served when means are provided to more clearly show the facts. In the examination of certain classes of forged writing an ordinary magnifying glass, enlarging only a few diameters, may answer, but the microscope with its corrected objectives and various adjustments is often necessary to show facts which, when thus shown, cannot be denied.

Strong suspicion is sometimes cast upon a document, or it may even be proven fraudulent by simply showing that a part of the writing was made after the paper was folded. An ink line crossing such a fold has certain distinct characteristics, but such a line may not be more than one one-hundredth of an inch in width, and the unaided eye cannot discover the fact, which under the microscope may be so evident that it must be admitted. A tiny portion of ink in such a case may actually have gone through the paper to the opposite side and under the microscope is unmistakable.

Fraudulent additions to documents, which it is claimed were a part of the continuous original writing, may be shown to have been written with a different pen or at a different time or under different conditions because the unshaded strokes, showing the width of the pen point as measured by the microscope with filar micrometer, show throughout a different average width as compared with the original writing. The microscope with this attachment enables even the unskilled

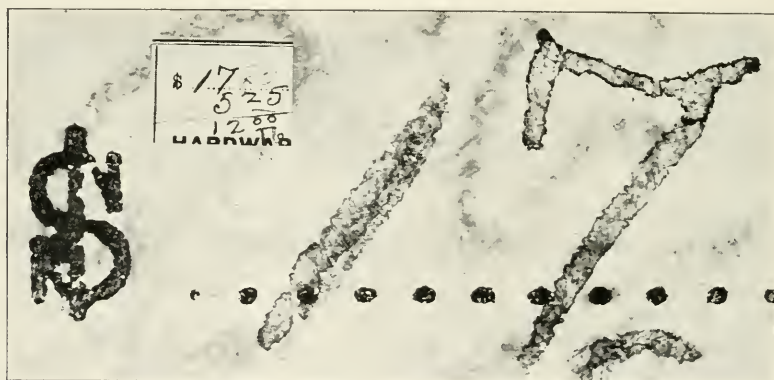


FIG. 1.—Raised Note Case. Numeral 1 in amount 11 changed to 7 by addition of stroke at top on one of several questioned notes. Transmitted light photomicrograph showing fraudulent addition as it appears as seen by the microscope.

observer to measure up to ten-thousandths of an inch, making comparisons possible which otherwise it would be utterly impossible to make.

Fraudulent additions and interlineations in documents often touch the signature or writing above which they are placed, and the actual sequence or order of writing is determined by showing which of the crossed lines was last written. This fact the microscope will often show with great distinctness, thus proving that the part in question was written after the document was signed.

Erasures and changes of a fraudulent character are often made in documents which, if examined only with the unaided eye, would escape detection, but which under the magnification of a good microscope any one can see.

Forged signatures are frequently first carefully outlined in pencil and then inked. The microscope shows the pencil marks not covered and the graphite caught in the ink film, and shows this with such clearness that the method by which such signatures were made is unmistakable.

Forged signatures are often not written but carefully and laboriously drawn

from a model with frequent liftings of the pen or with a stopping of its motion. Such lapping of lines and uneven ink distribution is shown by the microscope with astonishing clearness, especially under transmitted light observation.

Fraudulent documents are frequently brought forward, purporting to be several years of age, which in reality are only a few days or weeks old, and on which the writing fluid or iron nutgall ink still retains a distinct blue color and has not nearly reached its ultimate degree of blackness. A view of such writing under magnification in good daylight shows its exact tint, which, when made a matter of record may be compared with the tint and shade of the same ink at a later period when it may have turned entirely black, which would show conclusively that the document is fraudulent and not several years of age.

In the examination of questioned typewriting the microscope is necessary to show comparisons, measurements and numerous conditions bearing upon the genuineness and identity of such documents.

There are many other uses for the microscope in such examinations, but enough has been specified to show its importance. In many instances it is the compass that guides to the truth.

High power magnification in these various examinations is not usually required nor desirable, and the process of microscopic examination is very simple, and, with properly constructed instruments, judge and jury or any unskilled but intelligent observer can, with proper assistance, verify all testimony based upon microscopic examinations so that such

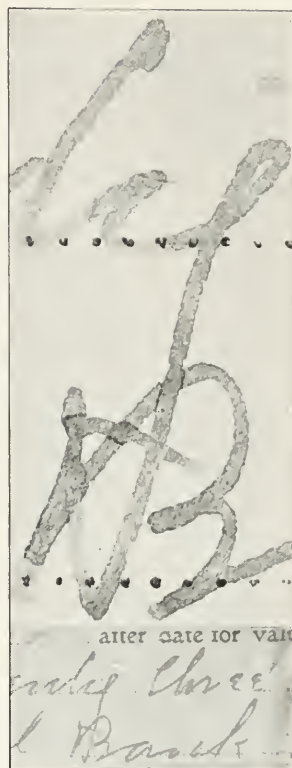


FIG. 2.—Crossed lines showing that amount in the case of a raised note was written after the name of the bank below it. Actual lines only  $1/100$  of an inch in width. Without the microscope the facts could not be shown.

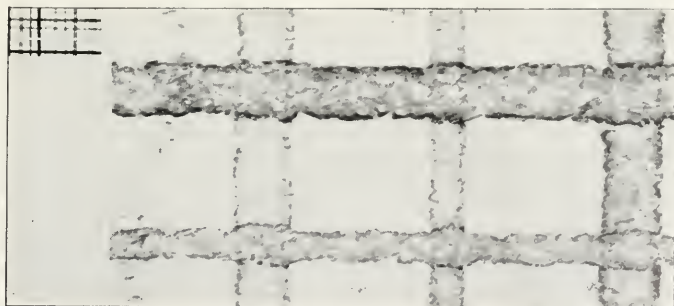


FIG. 3.—Crossed lines; vertical lines first made. Narrowest line  $1/150$  of an inch in width. Phenomena invisible without the microscope.

testimony is not a mere statement of opinion, which has but little weight, but rather a pointing out of significant facts.

Photo-micrographs of parts of questioned writings are frequently very useful, and in some instances are absolutely conclusive. Such enlargements show

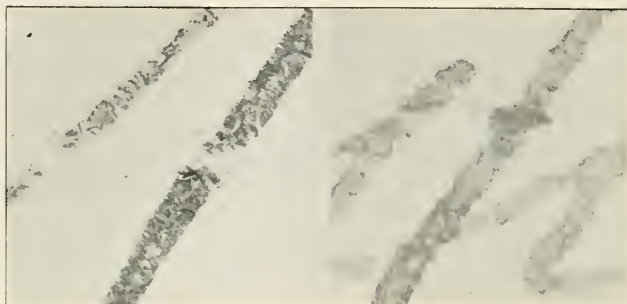


FIG. 4.—Ink lines across folded paper, also broken ink lines. Longer lines at the left preceded the folding. The line at the right followed the folding. Original lines about  $1/125$  of an inch in width.

plainly in permanent form what cannot be seen at all without the microscope. Perfect photo-micrographs of from twenty to fifty diameters' enlargement are easily made by using special photographic objectives and a regular camera, and such illustrations are easily verified by comparison with the actual image as seen in

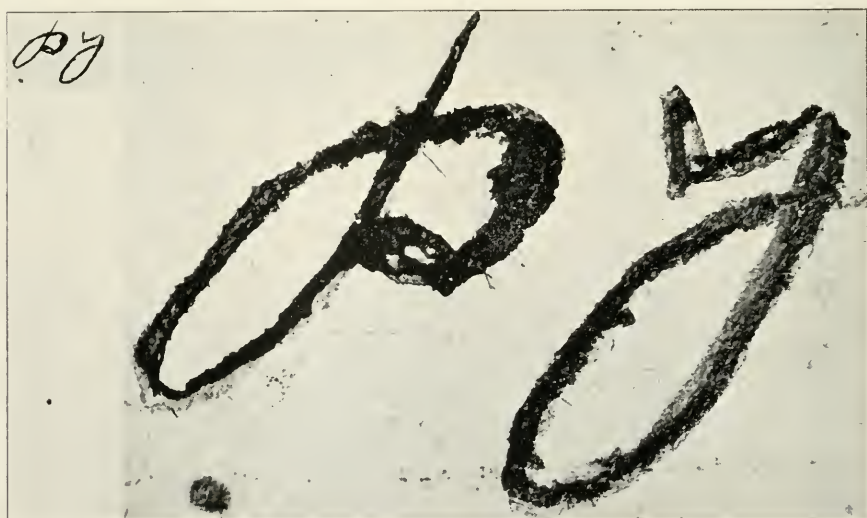
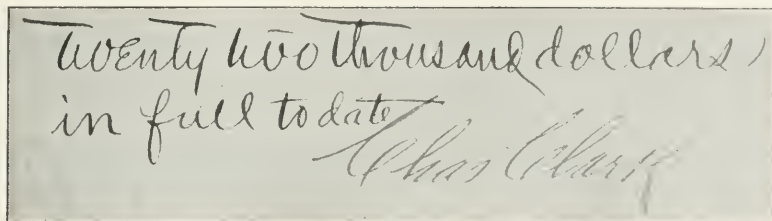


FIGURE 5. Retouched writing showing projecting edge of original line being part of a forged signature. Transmitted light photo-micrograph.

the microscope itself. For such work it is desirable that a camera should have a comparatively long bellows and an adjustable object board connected with the bed of the camera.



The microscope designed for the examination of disputed documents should have a large stage with ample room both at the back and in front of the objective, and such an instrument is greatly improved for the purpose if provided with a special large mechanical stage operated by rack and pinion. Such special microscope should also be provided with a sub-stage condenser for transmitted light examinations and a light converging lens on a movable arm for throwing additional light on opaque objects from above as required. A document microscope intended for miscellaneous work should be provided with several eyepieces and a variety of objectives. A one-sixth objective is as high power as is likely ever to be used, and this but seldom. A revolving nosepiece is a great convenience, and a polariscope is useful in some kinds of paper fiber examinations.



Twenty two thousand dollars  
in full to date  
Chas. Clark

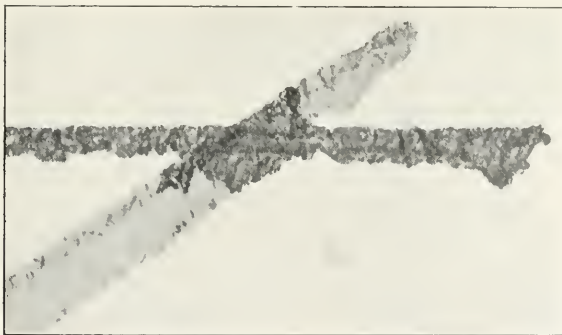


FIG. 6.—Crossed lines showing sequence of writing. The question to be determined being whether such a receipt was only for a definite amount or was "in full to date" when signed.

The Filar micrometer should always be included in such an outfit. It is very useful in many instances for readily making accurate measurements and comparisons, and but little skill is required in its manipulation. A camera-lucida attachment, by which drawings can be made directly from the microscopic image, is also very useful and affords an effective means for making illustrations and outlines, particularly of matter which may be introduced so late in the case as to prevent its being photographed. A camera lucida drawing board is of great assistance especially if work is to be done in court which is likely to be vigorously criticised.

For the examination of some kinds of disputed documents it is desirable that the microscope tube be mounted on a special stand without a stage and with an

open field directly under the objective. This permits the middle or any part of a large document to be examined microscopically, which otherwise would be impossible for lack of room to bring such parts into the microscopic field on the stage of the ordinary instrument. Such a special stand was devised for this purpose a number of years ago by the late William E. Hagan of Troy, New York.

The document microscope should be provided with rings having openings on the lower side placed back of the stage to hold a document of reasonable size when carefully rolled which will allow the edge or any part to be drawn out under the objective for observation. These rings obviate the necessity of folding, creasing or injuring the paper, and permit it to be reversed so that the image appears right side up. This is especially desirable when examination is to be made by those not accustomed to using the microscope, and who cannot properly interpret the reversed image.

Those unfamiliar with the microscope should understand when making a microscopic examination exactly what they are looking at and how much of it they are seeing. It is not usually understood that the image is reversed and



FIG. 7.—Pen Points : Ordinary Falcon ; Ladies' Falcon ; Broad Stub ; Ordinary Stub, and old corroded Steel Pen.

that the field of vision is restricted in proportion to the magnification. Many expect when looking into a microscope at writing to see the whole page greatly enlarged instead of a small portion of one letter or word. Observers should be seated, should not be hurried, and should take time enough to get the eye just at the proper point. Many look into a microscope and see nothing because they are not properly instructed.

If the examination does not require the determination of tints and shades of color, artificial light may be provided if necessary. Court room light is frequently quite dim and a good lamp placed in proper relation to the microscope may be of great assistance.

A special eye-shade attached to the top of the microscope tube to shield the eye not used for observation, is an assistance to those who are not accustomed to microscopic observations. To accommodate varying eyes it is also well to advise each observer to adjust the focus slightly by the fine adjustment until the object is perfectly distinct.

The amount of magnification required depends upon the question to be determined, but great magnification is not only not usually necessary but may actually

render the microscope entirely useless in showing the fact which is sought to be shown. It is an almost universal fallacy that the greater the magnification the better, the fact not being known or considered that the field of view is diminished in proportion to the degree of magnification. For examination of crossed lines, traces of pencil marks, line edges, paper fiber, retouching, and ink condition, quite high magnification may be useful, but for examination of writing as writing a magnification of from ten to fifty diameters gives the best results.

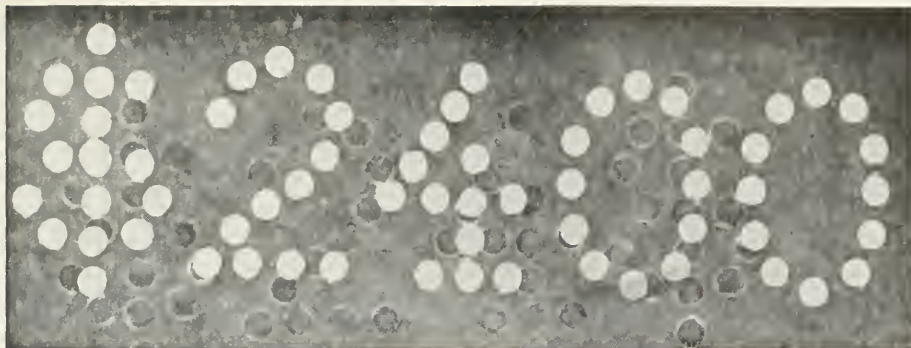


FIG. 8.—Check punch perforations filled in and larger amount punched out of same field. § 24 S changed to § 2400. Transmitted light view under magnification showing transparent rings around filled in portions.

Objections to the use of the microscope in court are based upon the somewhat natural but erroneous idea that what exists that is significant can be seen by unaided vision. Ordinary spectacles are simply lenses placed between the eye and the object looked at, by which means sight is corrected and improved, and the most elaborate and complicated microscope is nothing more than an extension of this principle. To be consistent, one who objects to the use of the microscope should also insist that judge and jury should be compelled to remove spectacles before examining a document that is questioned in a court of law.

Rochester, N. Y.

ALBERT S. OSBORN.

### An Absolute Alcohol Still.

In 1895, while working in the Anatomical Laboratory of the Johns Hopkins University, it occurred to me that the home manufacture of absolute alcohol would be more economical and satisfactory than buying it in small quantities. With permission from Dr. F. P. Mall, a small still was made which for a time gave satisfactory results. This apparatus consisted of an ordinary tin can and an inverted Liebig's condenser for the first portion of the work. When ready for distillation the condenser was changed to the inclined position and the alcohol was caught in an ordinary receiver. While this apparatus gave satisfactory results, it required more care and knowledge for its operation than can ordinarily be placed in an average janitor, already busy with numberless small chores. Since then, an absolute alcohol still has been constructed by Dr. Mall, which requires practically no care or thought on the part of the operator, yields an

excellent quality of alcohol, and is inexpensive. The apparatus is made of heavy copper, tinned on the inside, and a block-tin worm. It is composed of a gas-stove, an automatic water-bath, boiler, condenser, and receiver. (Figs. 1 and 5.)

The only especial requisite for the gas-stove is a stop-cock in the supply pipe by which the gas flow may be regulated, thus permitting the stop-cock in the gas main to be turned on full. A stove similar to No. 8157, Eimer & Amend's Catalogue, 1902, p. 211, is satisfactory.

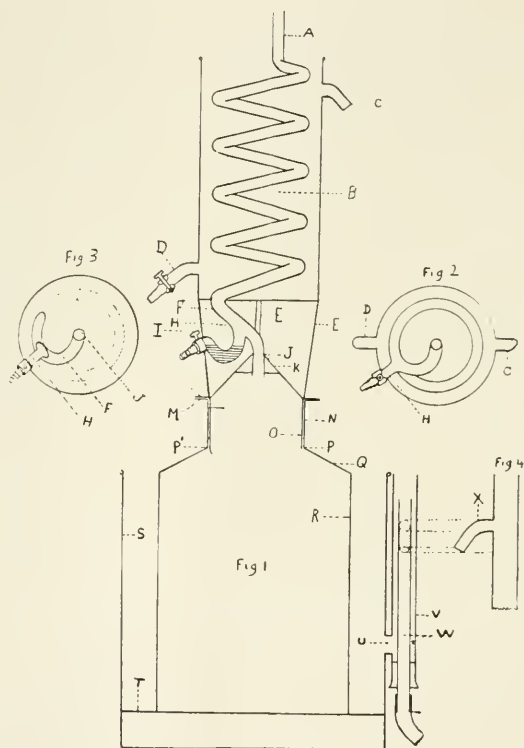


FIG. 1 represents median plane through still.

FIG. 2 shows relative position between tube H, D, and C. If the still is placed near or against a wall, tube H should be a little in front of the plane through D so that the cock I may be more easily reached.

FIG. 3 shows course of block-tin worm from bottom of condenser to J in Fig. 1, seen from below upward.

FIG. 4 is a side view of V, showing tube X.

Naturally, the size of the still must depend on the amount of work to be done. For making an amount of absolute alcohol used in our universities the following dimensions are sufficient: a water-bath, eleven inches in diameter and eleven and one-half inches high, with a substantial support one and one-half inches above its bottom to support the boiler and still, will allow one and one-half inches of water about the sides and bottom of a boiler eight inches in diameter and ten inches high. The support for the boiler should be fastened to the sides and bottom of the bath, with a small depression on the upper surface or several small uprights to hold the boiler in one place. The automatic water



supply now used on the ordinary water-bath in chemical laboratories is sufficient. The connecting tube U should be three-fourths of an inch in diameter and not longer than one-fourth of an inch, placed four inches above the bottom of the bath. The tube V should be one inch in diameter, extend from a point level with top of bath to a point one inch below lower side of tube U, and be well anchored to side of water-bath, near the top. Supply tube X, Fig. 4, sufficiently large to take a one-half inch rubber tube, is inserted at a right angle into the posterior side of tube V two inches from top. One-fourth of an inch from tube V tube X is bent downward at an angle of  $45^\circ$ , this is to prevent kinking of rubber tube connecting X with C. The height of the water in V is regulated by height of tube W, which is either fastened in V by penetrating a cork stopper or permanently fixed in a cap to screw on V. Tube W should extend to one inch below

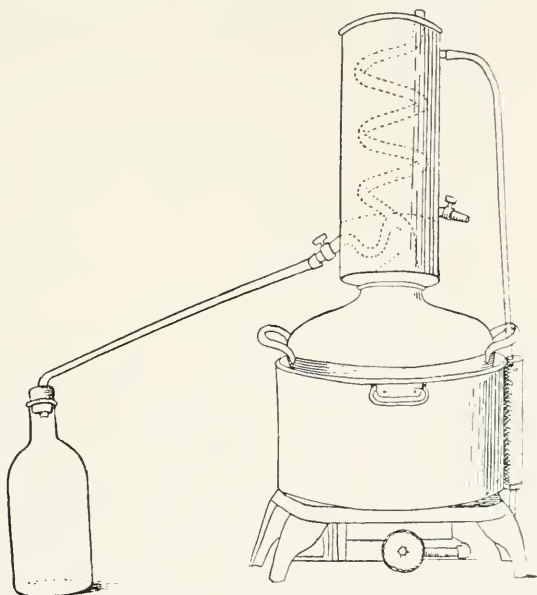


FIG. 5.—Side view of still, one-fourth scale.

top of V, and its lower end should accommodate a one-half inch rubber tube which carries waste water to a sink.

The boiler (Figs. 1 and 5) is eight inches in diameter and ten inches high from bottom to side Q, which may slope inward and upward at any desired angle. The opening P to P' should be at least four inches; walls of neck N may be parallel or slightly flared at top and two inches high. At top of N a heavy flange M three-fourths of an inch wide is firmly attached. The top is composed of wall O which closely fits into neck N and extends about one-quarter of an inch below the point P and P', with the lower edge slightly flanged inward as shown in the figure, to prevent, as far as possible, capillary attraction between the walls O and N. At the top of O a flange L, corresponding to flange M, is firmly attached, a sufficient distance above M to admit a rubber or asbestos ring. The top may then be firmly fastened to the boiler by placing several

simple clamps on flanges. Beginning at top of O, the wall K extends upward, cone shaped, to an apex J, through which passes the lower end of the block-tin worm A. This joint will be more secure if the tin tube extends about one inch below the apex and is braced as shown in Fig. 1. Immediately above the point J the tin tube bends at almost a right angle and runs in a spiral direction outward and upward to the bottom of the condenser B, the bottom of which should be about one and one-half inches above the point J. The spiral tube is shown in Figs. 2 and 3. In the middle third of tube F, Fig. 3, is soldered a short piece of tin tubing, in which a stop-cock I is placed, in such a manner as to form a small trap in the bottom of tube F, which must be filled before the alcohol, returning from the condenser, can flow back to the boiler, Fig. 3. This trap is made by removing the bottom and spreading the sides of tube F to receive tube H. Tube H, in Fig. 3, contains a stop-cock, which, when closed, causes the return alcohol from the condenser to fill the trap and flow into the boiler and when open permits the return alcohol to flow through tube H to the absolute alcohol receiver. The lower end of tube H is to receive a three-eighths rubber tube, which in turn connects with a glass tube the lower end of which penetrates a cork in the absolute alcohol receiver, which is an ordinary large bottle.

The worm should be of one-half inch block-tin tubing, the coils of which should be from one and one-half to two inches apart and three-fourths of an inch from the walls of condenser B. When the coil reaches the top of condenser B, it should be deflected until the wall of the condenser is reached, here securely fastened and then extend perpendicularly upward four or five inches. Condenser B should be at least five inches in diameter and ten inches high, and firmly attached to top K by four supports, E, E', E''. It is necessary to have condenser and top K one rigid piece to prevent straining the block-tin tube and disarranging the trap in tube F. Near the bottom of the condenser is a cold water intake O, for three-eighths rubber tubing, with stop-cock. On opposite side one inch from top of condenser is an outflow pipe, C, for a one-half inch rubber tube. One-fourth of an inch from condenser, tubes C and D are deflected at 45° to prevent tubing from kinking. Across the top of condenser run two bars at right angles for supporting a handle to which is attached a rope which runs over a small pulley fixed in a bracket a few inches above the condenser. By means of this rope and pulley the condenser is raised so that the wall O of top clears wall N of boiler and is then pushed to one side, so the boiler may be removed from the bath for cleaning and refilling. If the condenser is thus handled there is no danger of injuring the joint between O and N. The rubber tubing connecting the intake D with the water main and tube C with tube X must be sufficiently long to permit the condenser to swing free of the boiler.

The cheapest method of abstracting water from alcohol is by using fresh well burnt lime, quick lime, according to the formula  $\text{Ca O} + \text{H}_2 \text{O} = \text{Ca (OH)}_2$ , which when expressed in atomic weight values is 55.85: 17.95 or 3.11 grams of quick lime for each gram of water. Ordinary commercial 95 per cent. alcohol varies from 92 to 95 per cent alcohol, so contains from 5 to 8 grams of water per 100 cc. For safety it is best to calculate 5 grams of lime for each gram of water and allow for 10 c. c. of water per 100 c. c. of alcohol. On this basis it

requires 500 grams of lime per litre, or about four pounds of lime per gallon of alcohol.

Directions for operating the apparatus: The boiler is three-fourths filled with lime and 95 per cent. alcohol, placed in the water bath, tightly connected with the condenser and allowed to stand over night. On the following morning the bath is heated to, and kept at, about  $90^{\circ}$  to  $93^{\circ}$  C. During the first two or three trials the stove must be regulated by the stop-cock in the gas stove, keeping the valve at the gas main open full. When the gas supply has been regulated the valve in stove must remain untouched. The supply of water is regulated by the valve at D, keeping the valve on the water main open full. In this way when the apparatus is to be used instructions are to put — grams or pounds of lime and — quarts of 95 per cent. alcohol in boiler, tightly connect boiler and condenser, close valve I, allow to stand over night, in morning open water main valve and gas main valve full, light stove: in afternoon, shut off gas and water; next morning open water main and gas main valve full, light stove and open valve I. When alcohol ceases to flow into absolute alcohol receiver remove and tightly cork the absolute alcohol and immediately clean the boiler. If the lime is allowed to remain in boiler it may be difficult to remove.

Cost of absolute alcohol made in this way is about as follows. From 50 to 75 per cent. of 95 per cent. alcohol is recovered as absolute:

1 gallon absolute costs,	
2 gallons of 95 per cent., at 50 cents,	\$1.00
8 lbs. of lime at from 3 to 4 cents,	.24
About 100 ft. gas,	.15
Water, about	.10
	<hr/>
	\$1.49

The cost in 95 per cent. alcohol depends on the care in operating. If too much heat is used the loss will be greater. During the first day the alcohol need not boil, as a temperature near the boiling point will complete the reaction.

This still may also be used for making extracts, etc., and can be made of any desired capacity.

If the trap pictured in Fig. 3 be blown in a glass tube which could be connected with an ordinary Liebig's condenser a very convenient chemical apparatus would be had.

A number of these stills have been manufactured by Vaile & Young, 210 N. Calvert street, Baltimore, at a cost of about \$25 each.

Missouri State University.

W. J. CALVERT, M. D.

## Preparing Microscopic Plants and Animals for Student Use in the Laboratory.

Smear a glass slide with albumen fixative, as in preparing for the mounting of paraffin sections. Then place on the surface of the film of fixative a drop or two of water containing the forms which it is desired to stain. Let nearly all of the water evaporate by exposure to the air of the room until only the film of fixative remains moist. The slide can now be immersed in Gilson or any other fixing reagent, and then passed through alcohols, stains, etc., in the same way that mounted sections are handled.

I have had no difficulty in getting preparations of Paramæcium by this method, with very little distortion of the body, and any kind of staining desired. By this method students can prepare in ten minutes very satisfactory preparations of Protozoa for demonstration of nuclei, etc.

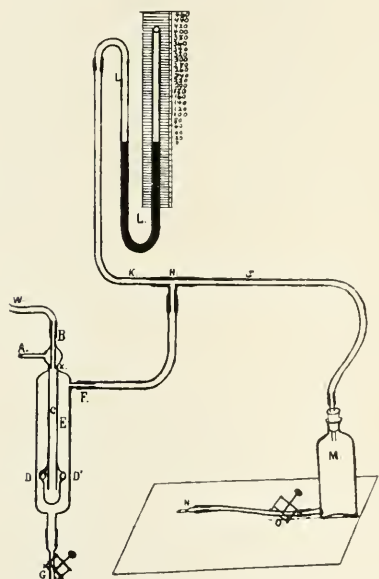
HARRIS M. BENEDICT.

University of Cincinnati.

## Fixation of Tissues by Injection.

During the past year, while preparing tissues for the course in histology at Johns Hopkins University, the injection method recommended by McFarland (*JOUR. OF APP. MICROSCOPY*, Vol. II, No. 10) of Leland Stanford, Jr., University, was given a trial. The result was so excellent as to lead to its continued use.

The method as suggested by McFarland is very simple. Two bottles with an outlet near the bottom are fitted up with tubes and clamps. This system is to be run up by means of rope and pulley to such a height as will give the gravity pressure required. Simple as the method is, it is very effective. It has, however, the disadvantage of being somewhat inconvenient to handle, and of giving no satisfactory registration of the pressure used, for the pressure varies not alone with the height of the injection flask, but with the specific gravity of the injection mass. Moreover, the method is such an excellent one that some of the more expensive fixing agents were experimented with, which would have been out of the question if one had to fill up an extensive injection apparatus, consisting of a flask and six or seven feet of rubber tubing, with so expensive a fixer as Hermann's fluid, for instance. Still further, it is entirely too difficult to keep so extensive a system



as warm as is necessary for a fine injection of blood vessels with carmine gelatin. Therefore connection was made, in the laboratory, with a very effective air blast run by water pressure, and this in turn gave place to the inexpensive apparatus figured below. As will be seen readily, the figure illustrates a blast apparatus. The tube W., leading from a water tap, is attached to a glass tube B. a part of the apparatus. This glass tube B. ends at x., a constricted portion of a chamber to which the air has free access through the tube A. Water passing through the system leaves the tube B. at x., drawing along with it a quantity of air into the tube C. The force of the water in this tube C. is broken by the cap at its lower end, and the water and air escape through the openings D. and D'. into the chamber E., out of which there are but two ways of escape, one at F. and the other at G. The escape of the water at G. is regulated by a screw clamp according to the air pressure desired. In the chamber E., the air, of course, rises to escape at F. through the rubber tubing connecting F. with the T tube interposed at H., through one arm of which connection is established, J., with the injection flask M., and through the other arm K. connection is made with the mercury manometer L.



It is obvious that in the chamber E., and in the tubes F. J. K. and in the flask M. the air pressure is the same, and will be registered on the millimeter scale placed back of the open leg of the manometer.

On this scale the reading is doubled, for the mercury falls in the closed as much as it rises in the open arm, and the difference between the columns is the sum of the rise plus the fall, or double the rise.

Let us suppose our animal, preferably a young one, killed, the artery exposed, and the ligatures in place ready for tying in the glass canula N. Let us suppose the injection flask filled with 300 or 400 c. c. of normal salt solution heated to 38 or 40° C., and the cork in place through which by means of a glass tube connection between J. and M. is established. Now loosen the screw clamp at O. just enough to let the 12-inch tube and the canula N. fill with the injection mass and drive out all the air. Now cut the artery, insert the canula and tie it in place. Then cut one of the large veins, say the inferior vena cava, to permit the washing out of the vessels, turn on the water at the tap and loosen the clamp at O. If only a little water is turned on, the water and most of the air escape at G. and the air pressure remains very low. Even if the full water pressure is turned on, if the escape at G. is too free, considerable air escapes that way and the air pressure remains low. Therefore, when a good flow of water is established, gradually tighten the screw clamp at G., at the same time watching the manometer; the water begins to back up in the chamber E. escape for the air at G. is cut off, and the air pressure rises. Thus a balance may be established between the water pressure used and the air pressure.

If the water in the chamber E. rises much beyond the openings D. D', the clamp at G. must be loosened a bit, or the water will back up into the tubes F. K. J. and then over into the flask M.

The limit of the pressure to be secured with this apparatus depends upon the water pressure, which, however, is usually much greater than should be used for injection. It is usually possible to get a pressure of 240 to 250 mm. mercury, i. e., a 5 pound pressure, and as a rule one-fourth of this is sufficient.

When the vascular system is well washed out by the normal salt solution, clamp off the flow at O., turn off the water at the tap, pour out the remaining normal salt solution, fill the flask as full as desired with the fixing agent heated to 40° C., and again begin the injection.

With this method any rapid fixer may be made use of. It is not always necessary to wash out the blood vessels with normal salt solution. Fixation is usually better, however, for having done so. With mercuric bichloride it does not so much matter. With formalin and Hermann's fluid, however, it is advantageous for fine fixation. The best fixers have proven to be mercuric bichloride, formalin, Hermann's fluid, and alcohol.

By this method the tissues, after a few minutes bath in warm normal salt solution, are instantly penetrated to the last cell by the fixing agent. The advantages are very great. The tissues, within a few minutes after anæsthetizing the animal (for anæsthetizing, illuminating gas is recommended as being cheap, and causing no salivation), are perfectly fixed in a normal position, at normal distention, and during normal activity. The possibility of post mortem

changes in the central nervous system is practically eliminated. The processes of digestion and absorption are arrested and fixed in the act, and tissues may thus be secured in any desired stage of physiological activity. Engorged tissues are caught with the blood in them, giving a picture of rare beauty. Blood and bone marrow are perfectly fixed.

For studying bone marrow the ribs of a kitten or baby rabbit fixed with  $\text{HgCl}_2$  will decalcify over night in a solution of 3 per cent.  $\text{HNO}_3$  made up with 67 per cent. alcohol, and from such ribs, sections  $3\frac{1}{3}\mu$  may be secured easily, giving a picture of bone marrow with its connective tissue framework normally distended and marrow elements in normal position. On sections so thin an oil immersion objective may be used, blood stains employed, and eosinophilic cells in great numbers may be demonstrated outside the blood vessels of the bone marrow. Particularly are these marrow elements, together with nucleated red blood corpuscles, shown with great beauty in the ribs of a 10 cm. embryo pig. Bone marrow in this form is a decidedly different tissue from bone marrow studied as a smear.

The thoracic wall of a small white rat, fixed by injection of Hermann's fluid, may be cut at  $3\frac{1}{3}\mu$  without decalcification, and, stained with iron hematoxylin, shows not only marrow elements, but the intercostal muscles and nerves in normal position with the usual beauty of a Hermann's fluid fixation. In short, we get a penetration with Hermann's fluid impossible by the ordinary method of using it.

There is scarcely a tissue that is not shown with new beauty by this method of fixation. Sections of lung fixed by  $\text{HgCl}_2$  injection and cut at  $3\frac{1}{3}\mu$  give a picture unequalled in beauty. The epithelial lining is intact and shows the more perfectly in that the tissue is at normal distention.

A brain fixed and hardened *in situ* presents a very different appearance from a brain supported on a sheet of cotton.

The method is invaluable not only in preparing tissues for classes in histology, but also as a research method.

In the use of  $\text{HgCl}_2$  as a fixer by the usual method, the crystalline deposits formed are very annoying and detract from the value of the fixer. This difficulty is overcome in the simplest manner. At a pressure of 130 mm. mercury, 400 c. c. of a saturated aqueous solution of  $\text{HgCl}_2$  are injected in about 10 minutes into a small kitten or rabbit, the time depending somewhat on the freeness of the venous opening. If the venous opening is not sufficiently free an œdema is likely to be caused, which in some cases is no disadvantage. Follow the injection of the  $\text{HgCl}_2$  by an injection of 500 c. c. of 67 per cent. alcohol. This not merely washes out the  $\text{HgCl}_2$ , but the  $\text{HgCl}_2$  is about 3 times as soluble in alcohol of this strength as in water, so the washing out is doubly effective and the hardening of the tissue is begun at the same time. After such a washing out, if properly done, one may cut out whatever tissues desired without blackening the knife or tissues. It is usually best to leave the tissues in 67 per cent. alcohol for one day, though, if necessary, they may be transferred at once to 82 per cent. alcohol after having been washed out with 67 per cent. alcohol.

It is found best to inject only one-half an animal at once. The canula

should be placed in the abdominal aorta with the mouth just above the cœliac axis when injecting the thoracic viscera and head and neck, and low down in the thoracic aorta when injecting the abdominal viscera. In either case the vena cava should be opened either above or below the liver. It is best to place a block under the back of the animal to insure a free venous outflow.

If the animal is rare or valuable, a double canula may be placed in the abdominal aorta and the whole animal injected at once. After such an injection the whole animal, or the part injected may be left over night in 67 per cent. alcohol and then removed to 82 per cent. alcohol, which should be changed a few times. Thus a great deal of tissue, excellently prepared in a very short time, may be had on hand for any emergency.

The inner ear of a guinea pig fixed by  $\text{HgCl}_2$  injection gives a rarely fine picture on section. After decalcification the cochlea should be laid open by a section passing through the modiolus. This permits better infiltration and imbedding, with the result that the delicate membranes are held in position by the celloidin when cut and do not present the appearance so often seen of having been dragged.

Tincture of iodine added to the 67 per cent. alcohol used in washing out the  $\text{HgCl}_2$  showed no noticeable advantage.

A very valuable use of the method is in the preserving of brain tissue. A brain may be fixed in situ by formalin injection, and then removed, the brain stem cut out and placed in potassium dichromate solutions preparatory to sectioning and staining by the Weigert-Pal method. Those who have had the disappointment of having such post mortem changes take place in the inner capsule and pyramidal tracts, before the penetration of the fixer, as to render the tissue useless, will appreciate the value of this procedure.

Though many uses of the method have been noted they are but a part of the many ways in which the method was found very valuable in the Anatomical Laboratory of the Johns Hopkins University during the past year.

Dept. of Anatomy, Indiana University.

BURTON D. MYERS.

DIFFERENTIATING COLONIES OF TYPHOID, COLON AND ALLIED BACTERIA.—Hiss has shown that it is possible by a very simple modification of ordinary culture media to differentiate the colonies of the typhoid bacillus and the colon bacillus upon ordinary plates. The media which he uses for this purpose are several in number. All of them, however, contain agar, and they are quite similar to the ordinary nutrient media. For example, the one upon which the largest amount of work has been done is made up of agar, 15 grammes; gelatine, 15 grammes; Liebig's extract, 5 grammes; dextrose, 10 grammes; water, 1000 c. c. This material, as will be seen, differs from the ordinary nutrient agar in hardly any point, except that the *peptone is omitted*. In the other media which he describes the peptone is in a similar manner always omitted. Using these media for cultivating typhoid and colon bacilli, Hiss finds the two species very readily differentiated. The typhoid bacillus produces a colony considerably smaller than the colon, and it also develops, after proper growth, an abundance of irregular filaments radiating from a central colony, whereas the colon bacillus produces a colony with a uniform outline. This filamentous condition is a very ready means of differentiation of the two types of bacteria.—*Journ. Med. Research.*

## Commendation of Worcester's Formol-sublimate-acetic Mixture.

This fixing fluid designed by Professor Worcester for the study of karyokinesis in teleost eggs, and lately described in this journal by Dr. Pearl, can scarcely be too highly commended for general work. For eight years I have used it more than any other fluid, and on account of its faithful fixing, its rapid action, and the good staining properties of the tissues fixed with it I consider it one of the most valuable fluids which we possess. It has the further advantage of being cheap, easily prepared and perfectly stable. I have used it for a great variety of objects, including a large number of Protozoa, Hydra, Lumbricus, and various other worms, mussel, crayfish, and the tissues of all classes of vertebrates, and have uniformly obtained good results with it. It has given better results than any other fluid on amphibian embryos and teleost eggs, and is excellent for the study of reproductive glands, ciliated epithelia, and for both chromatin and cytoplasm of dividing cells. The surface, form, and appearance of embryos is preserved with remarkable fidelity. The time of fixation and hardening may be very short, but tissues are not injured when left in the fluid for hours. They are less brittle than when fixed in Flemming's fluid, and seem to keep better in alcohol.

The failure of sublimate to fix cell membranes is corrected in this mixture (by the formol ?). When amphibian embryos are stained entire with borax carmine the cell outlines are of course indistinct. But if sections are stained with hæmalum and afterstained by means of a little acid fuchsin added to the strong alcohol used for dehydration, cell boundaries come out very clearly. This fluid is not to be recommended for nerve work. It does not fix medullary sheaths and apparently medullated tracts offer an obstacle to the fixation of other portions of the central nervous system.

J. B. JOHNSTON.

West Virginia University.

## A Model for Demonstrating the Structure of the Kidney Tubule.

It is usually difficult to explain by the use of figures alone the structure of the end of the kidney tubule and the relations of the blood vessels to it. For this purpose I have used for several years the model here described.

With clay, a model of the end of the tubule can be made by moulding a cylindrical portion with enlarged spherical end. This can then be cut away so as to show the character of the tubule, the relative thickness of the wall of the duct and the capsule, and the invaginated end. If now the middle of a piece of the flexible "cord" used for electric drop lights be held in the flame of a bunsen burner the insulation will be burned away and the constituent wires will separate, giving a fairly accurate model of the way an artery breaks up into capillaries and then unites again to form a vein. If desired, one end may be colored red and the other blue, to give the conventional arterial and venous colors. With this a glomerulus can be constructed and put in place on the clay model. On the side of the "vein" another bit of the insulation is removed to represent the capillaries around the secretory portion of the tubule.

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AARON L. TREADWELL.



## Effect of Various Hone-Stones on Edges of Steel Tools.

There appears to be no published record of systematic or thorough examination of the results of action of novaculites, whetslates, grindstones or other hones upon the edges of tools. The literature having any bearing in this direction seems to be limited to practical methods and processes in the application of hones. But it is evident that, in the preparation of tools not only for the mechanical arts but for microtome work, surgery, etc., the best results cannot be hoped for, in ignorance of the exact effects of hones of various materials and textures in their action on the edges of steel tools.

In order to obtain satisfactory results through a preliminary reconnaissance of this extensive subject—which is all that can be claimed for the work described in this paper—it seemed advisable to confine my attention to the edges of one simple kind of tool and to a limited series of hones. Through the Pike Manufacturing Company I was supplied with a duplicate series of flat-faced chisels of the same manufacture, form and size, honed with greatest care by an expert cutler.

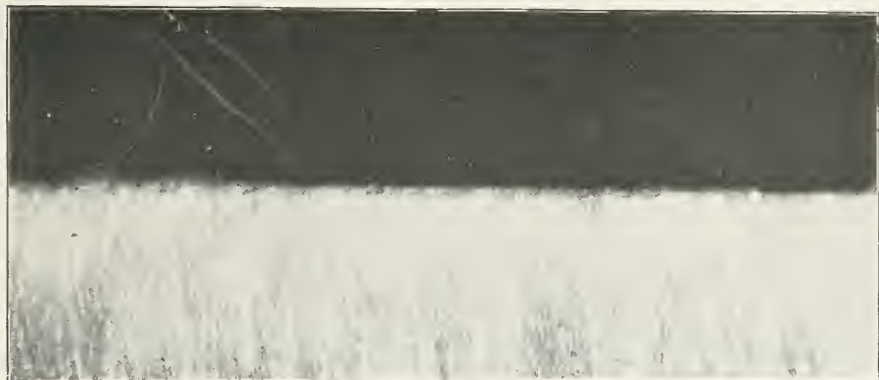


FIG. 1.

This was well done and thus eliminated all exaggeration of defects caused by rough or clumsy manipulation in application of the hones. The series of hones was restricted to three:

Hard Arkansas stone (A).

Lily-white Washita stone (W).

Medium India hone (I), this last an artificial hone prepared from corundum. These hones have been examined under the microscope and found to possess the following peculiarities of texture. The letters in first column correspond to the above three varieties.

<i>Hone.</i>	<i>Size of Grains</i> mm.	<i>Size of Cavities</i> mm.
A	0.01 – 0.03 ( $\frac{1}{2500}$ – $\frac{1}{830}$ inch)	Few: 0.01 – 0.02 ( $\frac{1}{2500}$ – $\frac{1}{1250}$ inch)
W	0.02 ( $\frac{1}{250}$ inch)	Many and connecting, sometimes of rhombic form:
I	0.18 ( $\frac{1}{40}$ inch)	0.20 ( $\frac{1}{125}$ inch) 0.05–0.15, averaging 0.11 ( $\frac{1}{227}$ inch).

As to the action of different hones upon the edges of tools, there is a current idea that it differs only or mainly in the amount of serrations produced along the edges, as seen in profile from one side. In evidence of this, figures have appeared in microscopical and other journals, exhibiting irregular saw-like teeth, *e. g.*, along edge of a razor, examined under a low magnifying power. This may be illustrated by a clipping from a recent journal: "A razor is a saw, not

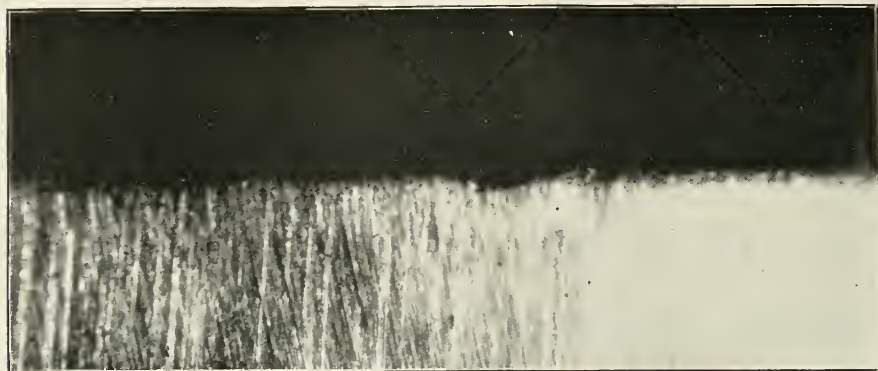


FIG. 2.

a knife, and it works like a saw, not like a knife. Under the microscope its edge is seen to have innumerable and fine saw teeth. When these teeth get clogged with dirt, honing and stropping will do no good. Dipping it in hot water dissolves out the debris from between the teeth."

In this case, with a magnification of 36, on the edge of a tool sharpened by the coarsest hone in this series (I), the results were obtained shown

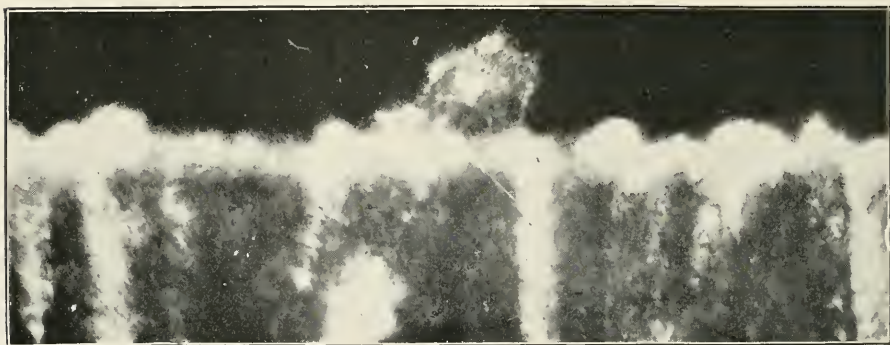


FIG. 3.

in Figs. 1 and 2. Doubtless in ordinary hands the serrations would have been larger and more irregular, but in this tool, after sharpening by an expert, the variations along the edge appear much too small to account for the known inferiority of the edge which can be secured by use of so coarse a hone. Evidently a far higher magnification was called for, and examination of the edges

from other points of view than profile. In default of these, all the manifest results were apparently inconsistent and anomalous, and pointed to a greater complexity in the problem than was at first anticipated. After some trials a magnifying power of about 280 seemed desirable to show all the important details. In the accompanying photomicrographs, the line of the edge some-



FIG. 4.

times shows, or, if taken somewhat obliquely, the center of the edge-band. Above this line a continuous wire-edge may appear (Fig. 5), or a remnant of it as a large grain near the middle (Figs. 3 and 4). Below the line the scratches of the hone are seen on the face of the chisel.

A little study of the edges also suggested that the thorough investigation of



FIG. 5.

the effects of each hone on the chisel edges would require examination of each edge on three planes or sections, *viz.*: vertical, horizontal and transverse.

#### I. VERTICAL SECTION,

*i. e.*, from side view of edge in profile, as when either flat side of chisel is held

up, with edge horizontal, on a level with the eye. The following observations were made:

*Hone A.* The surface of the edge (Photomicrograph, x 280, Fig. 3), held in that position, was found to be nearly plane and approximately horizontal, *i. e.*, level to slightly rolling. The projections of the cross-ridges or teeth, comparatively few, low and rounded.

*Hone W.* The surface of the edge shows a low wedge with central line, which in places sinks down to a level surface. In examining a considerable part of this surface, the teeth are found to be somewhat more numerous than with Hone A, and more irregular both in form and size.

*Hone I.* The surface of the edge was found to be somewhat rounded, with great irregularities, deeply scratched, rough, and occasionally indented, though level along short spaces. The teeth (Figs. 5 and 6) were many, angular, and irregularly scattered, sometimes becoming actual deep nicks in the edge.

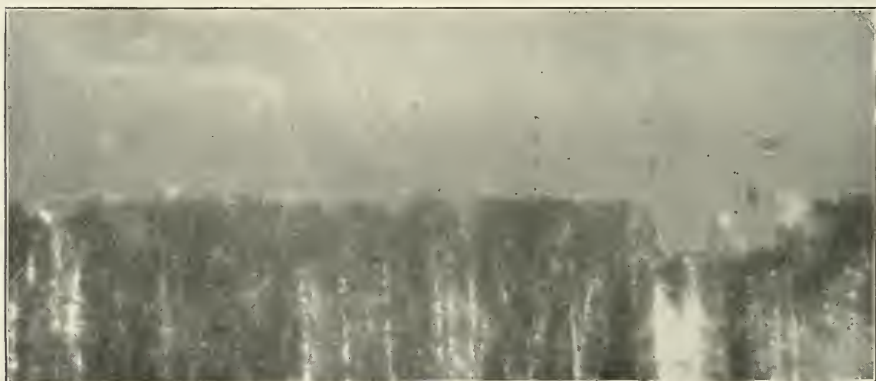


FIG. 6.

It may be necessary to add, in explanation, that under sufficient magnifying power, the so-called edge of a cutting tool, however keen, always displays, unless viewed exactly in profile, an actual flat, rough or rounded surface of varying breadth, according to the attrition during sharpening it may have experienced. In most of my photomicrographs it becomes visible, through a slight obliquity of position from the exact vertical section.

Any notable serration of edge, resulting from wear or from imperfect honing, is subject to objections which cannot be removed by mere honing. First, the increase of length of edge-line and, therefore of frictional resistance to penetration; this can be better appreciated by imagining an exaggerated serration by which the length of edge might be doubled, or more. Second, the increased tendency to form "wire" or "feather-edge" on honing a serrated edge. Third, the feeble penetration of the embayments or bases of the nicks, during cutting of material, and their tendency to accumulate refuse debris.

On the other hand, in tools used for a drawing movement, with some rapidity, on softer materials, a certain degree of minute serration in the edge may be adapted to corresponding inequalities of resistance in the material cut,



*e. g.*, in a razor edge for hairs of the beard, perhaps on exceedingly minute scale even in a microtome knife for alternations of cell-walls in tissues, or, on a larger scale, in a serrated bread-knife for a series of yielding gas cavities.

## II. HORIZONTAL SECTION,

*i. e.*, from top view of the edge, as when one looks down upon it.

*Hone A.* Surface of the edge (Fig. 3) appears as a nearly smooth, straight and quite uniformly narrow band, with an average thickness of edge of 4 microns ( $\frac{1}{6200}$  inch), varying from 2 to 7 microns.

*Hone W.* Surface of the edge (Photomicrograph, x 280, Fig. 4) shows a little wider band, often finely scratched, with a central line (that of the low wedge

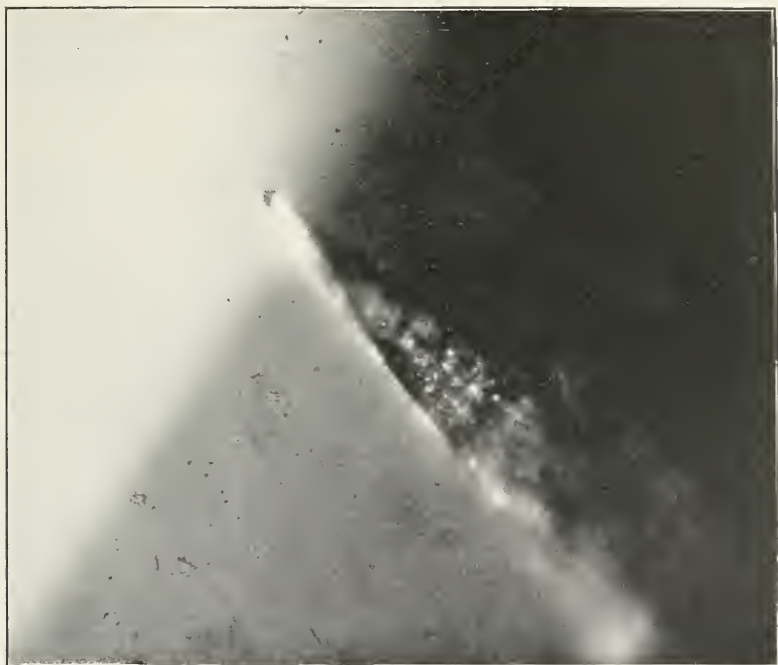


FIG. 7.

already referred to). It is slightly wavy from side to side in places, thickness of the edge averaging 6 microns ( $\frac{1}{16000}$  inch), varying from 3 to 9 microns.

*Hone I.* Surface of the edge roughly rounded to flat and well scratched, also often wavy, with some sudden indentations from one side or the other. Its breadth very variable, implying a thickness of the edge three or four times as great as with the other hones, averaging 15 microns ( $\frac{1}{17000}$  inch), varying from 4 to 23 microns. This edge surface is not shown in the photomicrographs, (x 280, Figs. 5 and 6), as they were made exactly on vertical section.

A little consideration will show, particularly as to the effects of Hones W and I, that the true or effective width of the wedge of penetration, in use of such a tool, is measured by a maximum thickness, that of the entire space that

lies between the extremes of divergence of the edge-band, as it waves or is suddenly bent in places from side to side (Fig 8). The differences of this virtual thickness of edge therefore much exceeds, with the different hones, those of the actual thickness above reported.



FIG. 8.

Again, this virtual thickness, or breadth of edge, ought to be adapted, in honing, to the size of cells or texture of the material to be penetrated by the tool. Thus, an edge like that prepared by Hone I would present a virtual breadth of at least 23 microns, and would tend to crush rather than to penetrate wood cells having a diameter of 11 microns, as in Willow, but would tend to penetrate cells of 28 microns, as in Ash.

### III. TRANSVERSE OR CROSS SECTION,

*i. e.*, from end view of the edge, when the line of the edge is directed toward the eye.

The actual ends of a chisel edge, however, have been so much rounded and worn, during grinding and honing, that they do not show a satisfactory cross section. This can be best obtained by nicking a chisel edge by sharp blow and mounting the fragment of edge, thus struck out, in such a way as to present the cross section to advantage. From such a minute splinter, a photomicrograph of the cross section was made ( $\times 170$ , Fig. 7), which shows the extremity of the cutting edge to consist of three parts:

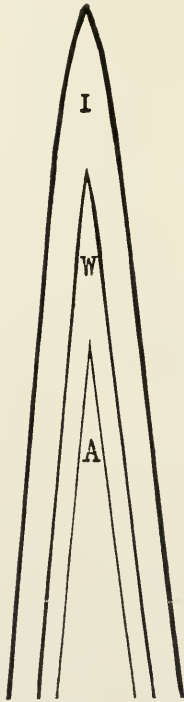


FIG. 9.

**CORE.** This is the wedge-shaped end of the interior material of the steel part of the chisel, and presents a very finely granular, crystalline mass, sparkling at the center of the wedge. Below that point its thickness is from 30 to 50 microns ( $\frac{1}{800} - \frac{1}{500}$  inch).

**LATERAL PLATE.** This is the dark coating of tempered steel on each face of the core, generally reaching about 38 microns ( $\frac{1}{600}$  inch) in thickness. It consists of finely fibrous metal, with fibres normal to faces of the core, so that a fine cross-fibration becomes apparent in the section, though not distinguishable in the photomicrograph.

**TERMINAL WEDGE.** The two lateral plates unite toward the edge-line to form this wedge, which, in the specimen photographed, was 83 microns ( $\frac{1}{300}$  inch) in length, from their junction to the edge-line. The contour of the cross section of this wedge, toward the edge-line, displays significant modifications with the different hones which can be

most clearly explained by means of a diagram (Fig. 9).

*Hone A.* This cross section has been presented in the photomicrograph,

Fig. 7, and displays a slender wedge, with nearly plane faces, with terminal angle about  $12^\circ$ .

*Hone W.* In this case, while the main faces of the wedge are inclined toward each other at an angle little exceeding that above stated, with Hone A, they terminate in two small faces which meet at the edge-line at about  $18^\circ$ ; this is the low wedge already distinguished on the vertical and horizontal sections. Sometimes a third set of planes occur; so that this cross section differs from that obtained with Hone A in the presence of inclined shoulders and in a somewhat larger terminal angle.

*Hone I.* The cross section of the wedge shows three or more irregular changes in the inclination of its faces as they approach the edge. The edge itself is rudely rounded, though sometimes nearly or quite flat, and the mutual inclination of the nearest planes may be  $22^\circ$ , in some places much more.

It is apparent that the angle obtained in honing should be fitted to the kind of tool and work in view; it may be large, as in a woodman's axe, or minute, as in the surgeon's scalpel or the knife of the microtometist. The advantage of strength, which accompanies a wide angle and series of shoulders, can be sacrificed only in the case of delicate tools of small angle, adapted for use with drawing motion, with little application of force, for penetration of soft tissues.

I am indebted to Mr. G. B. Waterhouse of the Department of Metallurgy, Columbia University, for the following pertinent facts concerning the microscopic structure and constitution of steel; and for an examination of the steel in these chisels:

"Tool steels, which contain usually over 1 per cent. of carbon, present on fracture a crystalline condition, the individual particles consisting of *pearlite*, covered by a thin brilliant film of *cementite*, that has been found to possess the definite formula  $\text{Fe}_3\text{C}$ . When a surface of such a steel is carefully ground, polished, and lightly etched with dilute nitric acid, it exhibits under the microscope a thin meshwork of brilliant white cementite enclosing the dark pearlite areas. A homely comparison is the honey-comb, the cell-walls being cementite, and the cells of honey pearlite.

"Under a high magnification power the pearlite is seen to be composite in its nature, a condition exceedingly well shown by steels that have been very slowly cooled from a bright red heat. This consists of an aggregate of thin plates of the definite carbide, cementite, interstratified with metallic iron.

"However, in the process of hardening, as the steels are raised past a dull red, at  $680^\circ\text{C}$ , the change or *recalescence* point, the pearlite areas become homogeneous, and form a new constituent, to which the name of *hardenite* has been given. To this constituent the intense hardness of quenched steels is due. As the heating is continued the cellular structure disappears, the cementite and hardenite diffusing one into the other; but after quenching in cold water it is found, in most cases, that a little cementite has again formed into meshes, notwithstanding the rapid cooling.

"Steels in this condition are intensely hard, being equal to No. 7, quartz, in Moh's scale, and are also very brittle. It is therefore the custom before use to

temper them or let them down, by heating to a lower temperature than was employed in hardening.

"In the case of flat chisels, containing about 1 per cent. of carbon, the quenched tools are carefully reheated to about  $270^{\circ}\text{C}$ , and again quenched, the right temperature being known by a polished surface showing a brownish-yellow oxidation film.

"The surface of a piece of one of the chisels, near the cutting edge, was carefully polished by means of successively fine emery papers and finally with wet rouge on a smooth cloth. After etching and examination with the microscope, no



FIG. 10.

structure could be distinguished, and this led to the conclusion that the superficial film was homogeneous, and probably broke with a conchoidal fracture. Then on another piece the surface was ground away, until the interior was reached, care being taken to keep the piece cold during grinding. After preparation and examination, it was found that it possessed a cellular structure (Fig. 10; magnification 220 diameters), the cells being composed of hardenite enclosing many very small globules of cementite; and the reticulated walls, of larger globules of cementite; the latter, while not touching, are so near to each other that they appear as continuous meshes under a low power."

The absence of distinguishable structure on what I have designated the



lateral plate has led Mr. Waterhouse to the conclusion "that the superficial film was homogeneous." By study of the side fracture of that film, however, under the higher magnification obtained by length of camera, I could readily distinguish the cross fibrillation to which I have referred; the superficial film which he studied was made up of the minute ends of these fibres. The core of the cutting edge is shown to consist of hardenite cells in a meshwork of cementite.

### RESUMÉ.

To sum up these observations in the reverse order in which they have been recorded, and so far as it seems safe to generalize from the limited series of experiments, the following appear to be the effects of successive application of coarse to fine hones to the edges of such a tool:

First, the effect of a coarse hone is to produce a roughly rounded thick edge, more or less deeply indented, waving along its line both vertically and laterally, and deeply scratched and nicked in places into rather angular teeth.

Its faces are also deeply scratched, the pair nearest the edge inclined at a wide angle, with several others below producing a series of inclined shoulders. Much of this irregular attrition has been undoubtedly caused by isolated grains, loosened from the coarse hone.

Second, the effect of a hone of medium grain is to produce a much thinner, straighter and more uniform edge, with finer scratches and serration. The faces nearest the edge present a low wedge, with smaller angle than before, and the secondary faces or shoulders occur in much smaller number.

Third, with a fine hone the results approximate the properties desirable in a wedge of most efficient penetration, viz.: only two, continuous, terminal planes at a small angle and without shoulders; a thin and nearly straight edge, so feebly scratched as to show but a minute and rounded serration and almost polished faces on sides of the wedge.

In conclusion, I have yet to refer to the surprising softness and flexibility of the true edge of the wedge, that part which reaches beyond its stiff core. It is illustrated by the sudden indentations and bendings noted on both the vertical and the horizontal sections, by the split shown at the extremity of the wedge in the photomicrograph (Fig. 7), and by the actual curling of the metal, observed in many cases under a light blow. This would probably indicate a loss of the rigidity or stiffness once acquired during tempering. Can this be due to the high temperature of the thin wedge, occasioned by friction, during the process of honing? If so, it may be suggested that it might be desirable, at least for tools of the higher class, worthy of the additional time and labor, to renew the original temper of the terminal wedge by a second and local tempering, after the honing has been completed.

It is also probable that the finish of the edge of a razor upon a strop or of a microtome-knife upon plate glass is effective, not merely by removal of remnants of feather-edge, but by straightening the line of the softened and flexible margin of the terminal wedge.

ALEXIS A. JULIEN.

## An Imbedding Medium for Brittle Objects.

I have tried various means of securing good serial sections of amphibian embryos. Aside from the collodion method, which is too tedious to be practicable, double imbedding in collodion and paraffin has been the most efficient of the methods known to me. The object is infiltrated in thick collodion and is then cleared in bergamot oil and imbedded in paraffin. It is better not to allow the collodion to harden by evaporation before clearing. When this method works at its best it gives good sections. The objections to it are that it is a slow method, that the hardening of the collodion in the bergamot oil is uncertain, that the cutting must be done with a very slow movement to get the best results, that the sections tend to roll or to wrinkle, and that ribbons are not easily made. At best the yolk cracks, breaks out, or crumbles away in a part of the sections.

In order to obviate these difficulties I have sought to mix directly with paraffin some substance which will play the part taken by collodion in the above process. It is necessary to have a tough, elastic substance, readily soluble in or miscible with paraffin, whose melting point is so different from that of paraffin that it will harden at a different time and so form a network throughout the tissue, the interstices of the network being filled with paraffin. The substance must at the same time be harmless to delicate protoplasmic structures and be adaptable to all the processes of paraffin work.

These conditions seem to be filled by ordinary caoutchouc or crude india rubber. The method is very simple. Mix with hard paraffin about one per cent. of india rubber cut into very small pieces. Dissolve by heating to 100° C. for twenty-four to forty-eight hours. Higher temperatures are to be avoided. Several days in the paraffin bath at 55° or 60° will serve the purpose. Undissolved rubber and impurities remain at the bottom of the vessel. Filter or use the supernatant fluid. Keep a stock of the prepared mixture cold, as the rubber separates out after a few weeks if the mixture is kept melted. Use exactly as ordinary paraffin, except that *xylol and not cedar oil must be used for clearing*.

The mixture (solution) has a melting point a little lower than that of the paraffin used, in accordance with well known chemical laws. It is satisfactory for all kinds of paraffin work. It may be cut at very low temperatures and makes ribbons better than paraffin. I have used it for amphibian and chick embryos and for brains and have been unable to discover any detrimental effect on the tissues. I have obtained better sections through the yolk of amphibian embryos imbedded in this medium than by the collodion-paraffin process. Wrinkled sections straighten well on water, as collodion-paraffin sections do not; but there is also less tendency to wrinkle. Tissues fixed in any way can be cut. It is not necessary to use a fixing fluid that will leave the yolk soft. Such fluids often do not fix other tissues well.

The hardened block is slightly brown, but does not differ perceptibly from paraffin in transparency. The melted solution is murky. This murkiness may be prevented by dissolving in the paraffin before the rubber is added enough "mineral rubber" (asphalt) to give the paraffin a light amber color. This paraffin-

asphalt solution has in slight degree the qualities desired in an imbedding medium for brittle objects. A more important quality is that it is more transparent than paraffin and so facilitates orientation of the object for cutting. Finally, the paraffin-asphalt-rubber solution combines the qualities of toughness and transparency, so that it will probably be the most valuable of the media described here.

The cost of either of these media need not exceed that of ordinary paraffin more than one cent per pound. I have not been able to test asphalt from different sources, but do not anticipate that there would be any practical difference. It remains to be determined by the experiments of others whether these media will injure delicate cytoplasmic structures. The small amount of rubber and asphalt used would not lead me to expect such difficulty.

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J. B. JOHNSTON.

## An Artificial Light for the Microscope.

During a considerable part of the year daylight is often insufficient for successful work with the microscope. Numerous contrivances for artificial illumination have been devised, some of them fairly good, but most of them thoroughly unsatisfactory. During the past two years experiments in artificial illumination have been conducted in the botanical laboratory of the University of Chicago, and as a result we now have a cheap and practical light. The idea is not at all new, since a somewhat similar device was used by Hooke more than two hundred years ago. Practically the same apparatus is now in use at Prof. Strasburger's laboratory in Bonn.

The apparatus consists, essentially, of a hollow sphere filled with liquid. A fairly good and practical light can be gotten with an ordinary lamp by allowing the light to pass through a wash bottle filled with a weak solution of ammonia copper sulphate. A piece of dark paper with a circular hole in it serves as a diaphragm and at the same time protects the eyes from the direct light of the lamp. Such an arrangement is shown in Fig. 1. Wash bottles, however, are not perfectly spherical and the mounting is not convenient. To secure a perfectly spherical globe, it was necessary to have a mold made. The globes, as we now use them, are of the finest flint glass, have a diameter of six inches, and are mounted in a convenient black frame, Fig. 2. The globe acts not only as a condenser, but also as a ray filter. For general laboratory work and for nearly all research work, a weak solution of ammonia copper sulphate has proved most satisfactory. The solution (to fill one six-inch globe) may be made by adding 50 c. c. of ammonia to 25 c. c. of a 10 per cent. solution of copper sulphate and then adding enough distilled water to fill the globe. If a white precipitate appears and makes the solution look milky, add more ammonia. The strength of the solution depends so much upon the power of the light that no fixed formula can be given. We simply dissolve in water a small crystal of copper sulphate—about as large as a grain of corn—then add about 50 c. c. of ammonia, and then add distilled water until a light, clear blue solution is secured.

With a very strong light, the solution may have a rather deep blue color ; with a less powerful light, the solution must be weaker.

In studying the extremely difficult achromatic structures concerned in nuclear division, a light violet solution of permanganate of potash is a good filter if the preparation has been stained in violet. Similarly, various filters may be used according to the staining of the more critical structures.

The Welsbach lamp furnishes an excellent light. It should be placed so that the rays will be focussed upon the mirror of the microscope. Some of the more

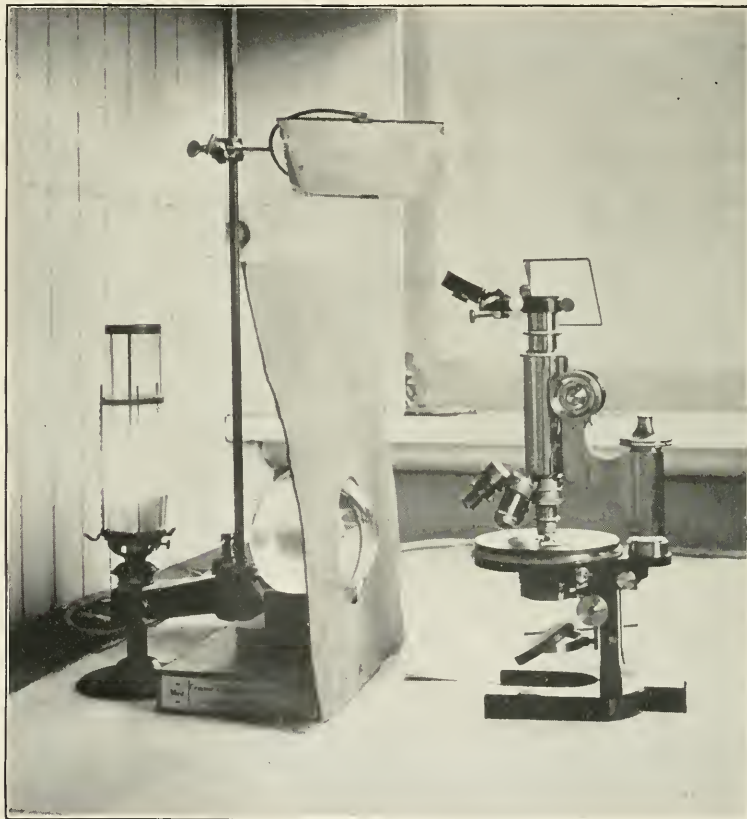


FIG. 1.

powerful acteyline bicycle lamps are quite satisfactory. The Argand type of gas light is good, but will usually need a reflector behind it. A kerosene lamp must also be reinforced by a reflector. The old fashioned silvered reflector, still used in country churches and halls, will do, but is hardly equal to the cheap reflectors of shorter focus which are so commonly used with incandescent electric lights. The incandescent electric light itself has not given satisfactory results. We have not tried the electric arc, but it would no doubt be satisfactory, if tempered by ground glass. It would probably be worth while to try the Nernst light.



When using the camera lucida, it is necessary to have a mirror placed so as to show a fairly strong light upon the paper and the pencil point. A piece of silvered glass three or four inches square is large enough. Such a mirror can be held by an ordinary ring stand, as shown in Figs. 1 and 2.

These globes are being used in our laboratory both for research work and

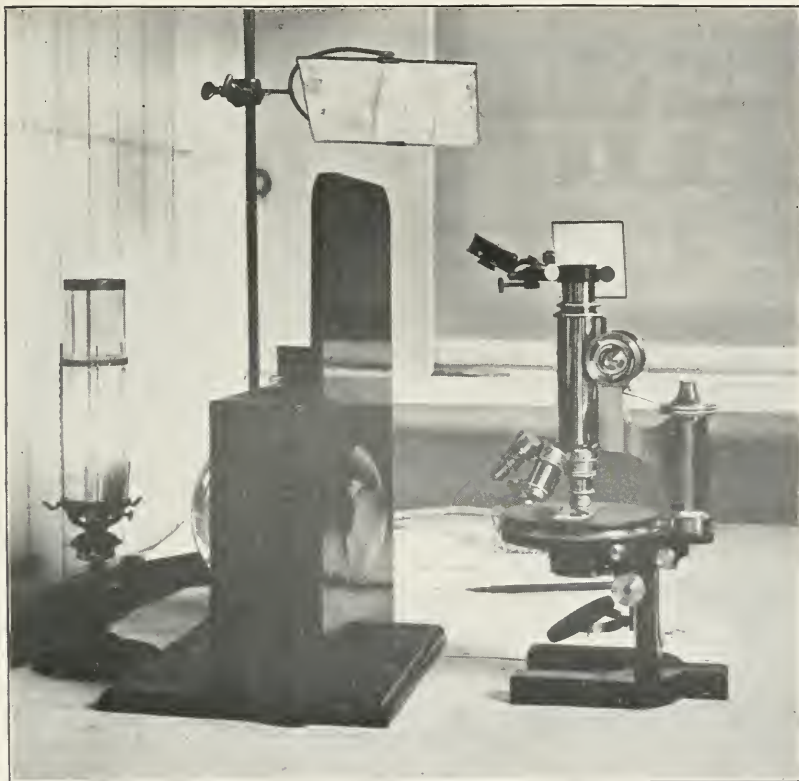


FIG. 2.

for ordinary class work.<sup>1</sup> For class work, three or four globes are used at a table and receive their light from a single Welsbach lamp.

We believe that such light is not only equal to the best daylight but is, in many cases, superior.

CHARLES J. CHAMBERLAIN.

<sup>1</sup> The globe and mounting which we are now using were made according to our directions by Dr. Wm. H. Knap, Powers building, 156 Wabash avenue, Chicago. Dr. Knap should be addressed for further particulars.

## A New Method of Affixation.

The method here described has given satisfaction to so many, whose difficulties with other methods have led them to try it, that we believe there are many more who would find it advantageous. Whenever serial sections, mounted on slides, are to be stained or treated otherwise, the chances of loss by washing off the slide are reduced to a minimum by this affixation. It is admirably adapted to affixing celloidin sections and has laudable advantages when applied to paraffin sections.

Three solutions are necessary: (1) Celloidin dissolved in oil of pennyroyal, oil of peppermint, or oil of cloves. This solution should have the consistency of very thick molasses. Several days are required for the complete solution of the celloidin. When clove oil is employed, we have what is commonly known as Shallibaum's affixative. (2) No. 1 diluted with four parts of synthol. (Columbian spirits, colonial spirits, or absolute alcohol may be substituted for synthol). (3) Chloroform in three parts of alcohol (95 per cent.).

Paraffin ribbons are dealt with in three ways. The thickness of the sections should decide, in part, which is most applicable. For very thin sections of soft tissues, it is sufficiently safe to mount on thoroughly cleansed slides, to flatten the sections with 70 per cent. alcohol by heat (55° C.) and make them adhere temporarily by evaporating the alcohol over a water bath at 40 to 50° C. Then remove the paraffin with xylol or benzine. With sections of greater thickness and having a tendency to be brittle, it is more reliable to use Mayer's albumen affixative as described by Lee (*Vade Mecum*). Flatness may be insured here also by heat, but 35 per cent. alcohol should be used to avoid the coagulation of the albumen. When it has evaporated, remove the paraffin as before. A third method of procedure is as follows: Smear the slides with No. 1 and press the sections down firmly with the fingers. Evaporate the oil in a warm oven at 40 to 50° C. When fairly hard, dissolve out the paraffin with benzine or xylol. It dissolves somewhat slowly through the celloidin. If the sections tend to break up and bits to float away in the xylol, it is well to brush over with No. 2 and let it evaporate, just before placing in the solvent.

When the paraffin is removed, sections prepared by all three methods are treated alike. Flow on No. 2 plentifully, by means of a large camel's hair brush, holding the slide in a slanting position so that the excess may be absorbed by filter paper. The xylol is thus replaced by the affixative. The slides should then dry for about ten minutes in a horizontal position. When dry the affixative is thick and sticky to the touch. If the sections have been made to adhere to the slide by No. 1, one must proceed with great care at this stage. No. 1 is readily dissolved by No. 2 and the sections are apt to float about. Hence get rid of as much xylol as possible with filter paper and flow on No. 2 with a single stroke of the brush. Quickly place the slide in a horizontal position to dry. When dry, brush over again with No. 2, spreading the affixative over the ends, edges, and back of the slide and dry again.

The slides are now out of danger. The oil is removed by placing in No. 3,

the chloroform of which dissolves the oil and at the same time hardens the celloidin. After three to five minutes the slides may be carried to stains, whether they be alcoholic or aqueous, without fear of losing a section. If the chloroform does not act long enough the whole series will float off, after soaking in water. The sections will still be held in a film of celloidin and may be rescued by floating back onto a slide, dehydrated by dropping alcohol (95 per cent.) over them and finally affixed by flowing No. 2 over them again.

Methylen blue, fuchsin and anilin blue-black stain celloidin, hence these should not be used with this affixation.

After staining, one may carry the sections up through the alcohols to 95 per cent. Absolute alcohol must be avoided unless chloroform has been added to it. It has been found most advisable to clear from 95 per cent. alcohol, by using cedar oil, turpentine, oil of cajeput, to which a little chloroform has been added, carbol xylol, creosote, or anilin oil. Oil of cloves, pennyroyal, peppermint and bergamot are good clearing agents, but require chloroform to keep them from dissolving the affixative. After clearing, run to xylol and mount in Canada balsam.

When celloidin sections are to be mounted, they are arranged on the slide in 95 per cent. alcohol. After draining off the excess of 95, solution No. 2 is applied freely with a camel's hair brush. When dry it is hardened in No. 3 and the subsequent processes are carried out as above directed for paraffin sections.

Science Teacher, Little Falls High School, Little Falls, N. Y.

C. W. HAHN, A. M.

## A New Screen for the Projection Microscope.

In fitting a lecture room with a projection microscope, we found that the heavy curtain hung on spring rollers, that is so much used for projection purposes, was unsatisfactory, and since a sufficiently large area of white wall, which gives the best surface for this work, was not available, we have spent some time in devising a satisfactory substitute. The college janitor, Mr. Downing, has constructed a screen which gives thorough satisfaction, and is, so far as I know, of an entirely new design.

Directly across the room from the lantern is a blackboard, built into the wall, with chart frames sliding in grooves at either end, and so balanced that they can be drawn down in front of the blackboard or pushed up over it, out of the way. Another frame, eight feet square, was made and hung so as to slide exactly as do the chart frames. On this was fastened, with glue and tacks, large sheets of very heavy cardboard. Their edges were fitted as carefully as possible, and any spaces left filled with putty, and the joints painted over with shellac. The whole was then covered with several coats of white kalsomine spread very thin, and of such a composition that brush marks are not noticeable. The result is a surface that is almost as good as a white wall, and much better than any curtain I have ever seen. When in use the screen is drawn down in front of the blackboard, and later pushed up above it, entirely out of the way.

Our lantern is a Thompson double one, the upper arranged for ordinary lantern slide projection, the lower for use with the microscope. The upper lan-

tern rotates on the lower, so that two pictures may be thrown at the same time upon screens placed side by side. We have a curtain for use with the upper lantern, but the screen just described is so much more satisfactory that we use it altogether, except where both are needed at once.

The frame for the screen must be well braced, to guard against warping, for the cardboard absorbs so much moisture from the kalsomine that it exerts a good deal of a pull on the frame. Evidently, also, the larger the sheets of cardboard the fewer will be the joints and the smoother the surface. The sheets we used were bought originally for chart making and were about  $2\frac{1}{2}$  by  $3\frac{1}{2}$  feet. This made an excessive number of joints, but the kalsomine covers them so well that they are not noticeable when in use.

AARON L. TREADWELL.

Vassar College.

### Mitosis in Root-tip of Hyacinthus.

The several species of the cultivated Hyacinth offer very good material for the study of indirect nuclear division, in that their roots are mostly large and grow rapidly. Water cultures grown in a hothouse are especially to be recommended, and the young roots are to be cut off close to their point of emergence from the root-bearing area of the bulb, when they have attained a length of about one-quarter inch, as they are then in a most active stage of growth. A good plan also is to allow roots to grow for some time and then to cut off all except a few that are just coming through; these latter then grow very rapidly and are eminently suited for the preparations required for the study of mitosis.

For this purpose, the roots are hardened and fixed in Flemming's solution (I have found the following strength very suitable for hyacinthus: osmic acid .1 per cent., chromic acid .1 per cent., glacial acetic acid .2 per cent.). The roots remain in this for a day and are then washed in distilled water and subsequently transferred to pure spirit (methylated). From this material it is easy to cut very thin longitudinal sections, about one cell thick, by hand, the root being fixed in split pith. This method is certainly quite as good as paraffin embedding and does not disorganize the cells so much.

For staining, I can recommend single-staining, either with acetic methyl-green or concentrated aqueous solution of gentian-violet (Grübler's). Double and triple staining gives good results, but if safranin is used, it should be in dilute aqueous solution and the sections should not be allowed to remain in it for more than 5 minutes. Before staining at all it is advisable to treat sections with 5 per cent. solution of hydrochloric acid, in order to dissolve out the saphides which exist in certain cells of the section; then wash them. With these precautions very good results may be obtained, the sections being mounted as usual in xylol-balsam. All stages in mitosis may be found in a section, and the preparatory stages are particularly fine as also are monastes figures. The disastes phases are not quite so clear, owing to the large number and closeness of the separate loops, but in some cases distinct loops can be made out and counted.

The formation of the cell-plate is very clear and the separate thickenings on the spindle-fibrils are quite distinct. I could not find centrosomes, but this is probably on account of inadequate staining processes; if present, they are exceedingly small, although the nuclei are relatively enormous when compared with those of other plants, even *Fritillaria*. I may mention that, amongst the *Liliaceæ*, *Hyacinthus* and *Fritillaria* are perhaps the two best for the study of mitosis.

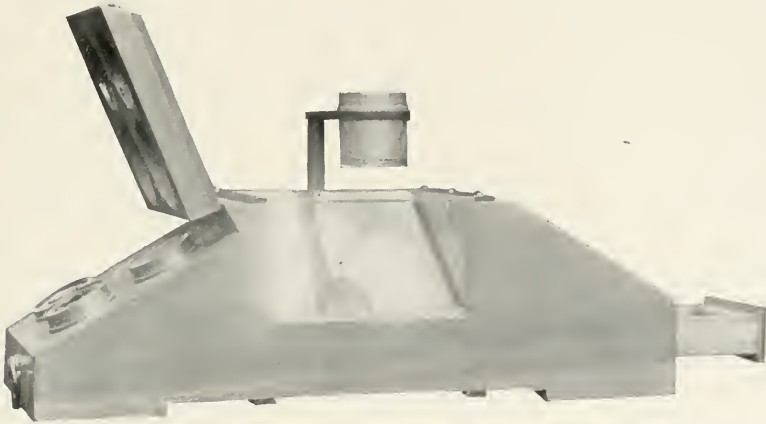
HARRY A. HAIG.

London, England.



### A Barnes Dissecting Stand.

The accompanying photograph of a Barnes dissecting stand illustrates some modifications which I recently had made, and which I find add considerably to its value. The holes for the lenses are of such a size that they fit snugly, and the hinged cover may be fastened with a hook, thus keeping the contents securely



in place. The drawer holds teasing needles, forceps, and camels hair brush. While this stand is somewhat more expensive than the usual form, I find that its greater convenience amply compensates for the increased cost.

Textile Laboratory, Western Electric Co.

CHARLES E. M. FISCHER.

### On the Use of "Sea-Lettuce" (*Ulva*) in Orienting Small Objects for Sectioning.

The various methods of orienting small objects for sectioning may conveniently be classified into three groups ; (1) the methods of orientation by sticking objects on a thin plate before embedding, (2) those of orienting objects in melted paraffin, and (3) those of orienting objects after embedding. As the second and third groups do not concern us here, I shall briefly mention what methods belong to the first. This group again includes three classes, according to the period at which the object is fastened : (A) Fastening the fresh object by means of a fixing fluid with or without albumen fixative, (B) fastening the object preserved in alcohol by means of albumen fixative and alcohol, and (C) fastening the object after being clarified by means of Schällibaum's fixative.

Under A, I may mention Häcker's<sup>1</sup> and Schydowski's<sup>2</sup> methods. Häcker used the following method in embedding the egg of *Myzostoma*. The eggs are placed with a very little water on a piece of *Ulva* spread on a watch-glass ; then

<sup>1</sup> Häcker, V. Praxis u. Theorie d. Zelle u. Befruchtungslehre, p. 111, 1899.

<sup>2</sup> Schydowski, A. Zeit. wiss. Mikr. Bd. 13, pp. 200-204, 1896.

they are killed by a fixing fluid, which at the same time sticks the egg to the alga. The objects are in this process not oriented, yet this method can profitably be used for our purpose. Schydrowski recommends a thin plate of photoxylin to stick objects on. Under B, I can mention only Samter's method.<sup>1</sup> In this the shell membrane of a hen's egg is used as substratum, and fish-glue as the fixative. Under C, we can consider Patten's simple but excellent method,<sup>2</sup> and its modifications by Woodworth<sup>3</sup> and Hoffmann<sup>4</sup>. Patten and Woodworth use a slip of writing paper ("linen cloth") to fasten the object on, while Hoffmann prefers a small piece of glass. Lately Drew<sup>5</sup> has improved Patten's method in first draining off the superfluous oil on an inclined plane of tracing linen. Jordan<sup>6</sup> fastens the objects on a movable metal sphere coated with resin. A gelatine plate is used in Field and Martin's complicated method<sup>7</sup>. Apathy<sup>8</sup> also uses a gelatine plate to give a guide line on the celloidin block, although he does not stick the object on the gelatine plate. Halle<sup>9</sup> recommends for the same purpose a plate of hardened white of egg. There may be other similar devices which are not as yet made public.

The method I am about to describe is simply a modification of Patten's method, in which *Ulva* is substituted for writing paper. The reason I prefer the alga to paper, we shall see later. My present account is, therefore, mainly a repetition of Patten's, yet I think the *Ulva* method, described at length, will be found of use by the reader who is not familiar with this line of technique. There are two things which should be at hand before orienting small objects by this method; one, a small piece of preserved *Ulva*, the other, celloidin-clove-oil.

*Ulva* can be obtained readily in any quantity between tide-marks, as a "by-product" of summer collections. The alga should be kept in 70 per cent. alcohol until all the chlorophyll comes out. By changing the alcohol several times this will be quickened. After being bleached the *Ulva* fragments should be stained slightly with borax carmine. A small rectangular piece is cut out from the stained *Ulva*, the size depending upon the number of objects to be arranged on it. After a *thorough* dehydration in absolute alcohol the pieces are clarified by adding xylol drop by drop, and are preserved in clove oil. It is advisable to have at hand many of these pieces of various sizes ready for use.

To make the solution of celloidin in clove oil a vial should be partly filled (about one-fifth) with granulated celloidin and then completely filled with clove oil. The vial is placed on the paraffin bath for a few hours and shaken several times during heating. By doing so, not only is solution of the celloidin accelerated, but the water in the clove oil, if present, is also driven out.

Just before imbedding, a piece of *Ulva* prepared as above is taken out and pressed between the thumb and finger until it becomes perfectly flat. The *Ulva*

<sup>1</sup> Samter, M. Zeit. wiss. Mikr. Bd. 13, pp. 441-446, 1897.

<sup>2</sup> Patten, W. Zeit. wiss. Mikr. Bd. 11, pp. 13-15, 1894.

<sup>3</sup> Woodworth, W. M. Bull. Mus. Comp. Zool. Vol. 25, pp. 45-47, 1893.

<sup>4</sup> Hoffmann, R. W. Zeit. wiss. Mikr. Bd. 15, pp. 312-316, 1899.

<sup>5</sup> Drew, G. A. Zool. Anz., Jg. 23, No. 611, pp. 170-174, 1900.

<sup>6</sup> Jordan, H. Zeit. wiss. Mikr. Bd. 16, pp. 33-37, 1899.

<sup>7</sup> Field and Martin. Zeit. wiss. Mikr. Bd. 11, pp. 11-12, 1894.

<sup>8</sup> Apathy. Zeit. wiss. Mikr. Bd. 5, p. 47, 1888.

<sup>9</sup> Halle. Zeit. wiss. Mikr. Bd. 12, p. 365 (Referat), 1895.

is placed on a ruled slide (*vide infra*) under a dissecting microscope. A *very little* celloidin-clove-oil is smeared on the alga and the object, clarified with clove oil<sup>1</sup>, is laid on it. Care should be taken to transfer as little oil as possible with the object. Sometimes Drew's method of draining off the oil is found very useful, but for this purpose tracing linen is not necessary, a glass plate or a piece of glazed paper answering just as well. Now as to orientation. Two cases may be distinguished; there is a certain kind of object in which orientation in only two dimensions or axes is necessary. To this belong rather flat or elongated objects like a *Nereis* larva of three segments. There are, on the other hand, other objects in which orientation in three axes is required; these often have these axes of nearly equal length, as in a *Polygordius* larva with but slightly elongated post-trochal region. In the former case all that is necessary is to move the object to make one of its axes parallel to one edge (the guide-edge) of the *Ulva* piece. Then a drop of xylol or cedar oil is poured on the object, which fastens it firmly to the *Ulva*. The slide is placed under a compound microscope and examined whether the object is correctly oriented. If not, the *Ulva* is cut in definite relation to an axis of the object. In the second case, when orientation in three dimensions is necessary, the object is first moved by means of a needle until one of its axes comes into a plane perpendicular to the *Ulva* surface. The subsequent processes are the same as in the first case. But quite often objects of this kind cannot be kept long enough in a desired position. In such cases it is better to drop xylol or cedar oil on the object as soon as one of its axes comes in a vertical plane, and subsequently to cut the *Ulva* so as to make one side (the guide-edge) parallel to the desired plane of section of the object. As before, the piece should be examined with a higher power, and the orientation controlled. The two corners opposite to the guide-edge (Fig. A. a. and b.) are then cut off in order to avoid confusion between the guide-edge and the

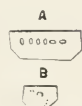


FIG. A.—Pieces of *Ulva* with objects oriented on them. The guide-edges are here represented by thicker lines.

others during the following manipulations. (In the figure the guide-edges are represented by thicker lines.)

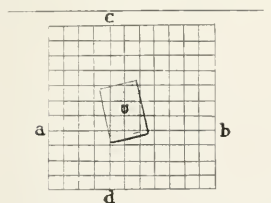


FIG. B.—Piece of *Ulva* with object on it placed on a ruled slide.

Here I shall describe a little device for cutting the edge of *Ulva* exactly parallel or perpendicular to one of the axes of the object which has been fastened on the alga. The *Ulva* piece is placed on a ruled slide<sup>2</sup>, *i. e.*, a slide having two sets of parallel lines, say 2 mm. apart, intersecting one another at right angles, as is shown in Fig. B. The *Ulva* is first moved so as to make an axis of the object fall on one of the parallel lines of the slide, and then cut

<sup>1</sup>Since prolonged stay in clove oil is rather injurious to the objects, it is always better to use cedar oil first for clarification, and just before orientation to place the object in clove oil for a few minutes. In this way shrinkage is prevented to a great extent. Cedar oil should be used, especially when the object is to be studied *in toto* before sectioning, since in this the object can be kept indefinitely without danger of becoming brittle (Cf. Bolles Lee. A. Zool. Anz., 8 Jg. p. 563, 1885, and Vade Mecum 5th ed., pp. 81, 97; Hoffmann, R. W., Zeit. wiss. Mikr. Bd. 17, p. 446, 1901).

<sup>2</sup>The ruled slide can be had from the Bausch & Lomb Optical Co. This slide may be used for other purposes, *e. g.*, measuring small objects when used with an ocular micrometer, counting the number of small organisms and arranging objects in regular rows for total preparation.

along any other line parallel or perpendicular to the axis (a b or c d in Fig. B). When more accurate orientation is necessary, an ocular micrometer, ruled slide, and mechanical stage should be used (Fig. C). The eye

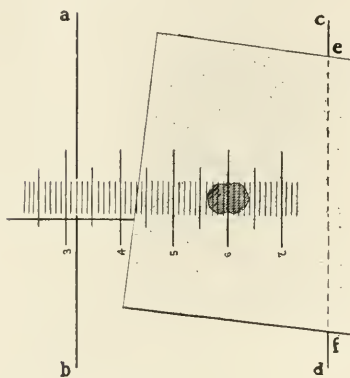


FIG. C.—The egg and the lines of micrometer are drawn with the camera (x70) while the *Ulva* piece is diagrammatically reduced. *a b* and *c d* represent two (not adjacent) lines of the ruled slide, the distance between these two being also greatly reduced on account of the lack of space.

piece is first rotated until the lines of the micrometer become parallel to those of the ruled slide, the *Ulva* piece then moved so as to make the axis of the object coincide with a line of the ocular micrometer, and finally cut along any line of the slide, *e. g.*, *e f* of Fig. C.

Now let us come back to the subsequent processes. The *Ulva* on which the object is correctly fastened, is placed in xylol or cedar oil for a few minutes and then imbedded in the usual way. It may be found convenient to imbed the *Ulva* in a drop of paraffin, and mark the direction of the guide-edge on the drop, or the *Ulva* piece with the objects on may be preserved in cedar oil, until the time of imbedding. In this way the loss of very small objects is prevented, especially in transportation. The paraffin block containing the *Ulva* is trimmed off from the side on

which the objects are not attached, leaving a very thin layer of paraffin over the guide-edge. The block is then set on the carrier of the microtome so as to make the guide-edge parallel to the knife and the *Ulva* piece vertical, with the aid of a hand-lens. This is of great importance; for, if this be neglected, the orientation of the object will be lost. After the block is rightly set the guide-edge is trimmed until the object appears just beneath the surface. The sections are cut without paying attention to the object itself, and when mounted the objects will be found cut exactly through the desired axis.

The *Ulva* method, as we have seen, is very simple, and a few trials will convince one of its accuracy. Moreover, it seems to have a few advantages over Patten's method: (1) the transparency of the *Ulva* helps orientation of small objects under a fairly high power, (2) in using *Ulva* it is not necessary to pull the substratum off from the paraffin block, and consequently any amount of celloidin-clove-oil can be used according to the nature of the object, and (3) not only does the *Ulva* not interfere with the sectioning, but the sections of *Ulva* serve as guide-lines in reconstruction.

In conclusion, I may mention another important use of celloidin-clove-oil described by Patten. In mounting small objects, it often happens that they slide out toward the margin of the cover-glass, or, if not, they will not stay in the desired position. In these cases, celloidin-clove-oil followed by xylol keeps them in any desired place and position. Celloidin-clove-oil is, therefore, often indispensable in mounting in regular order many small objects under one cover-glass, *e. g.*, developmental stages of annelids and echinoderms, appendages of small crustaceans.

N. YATSU.

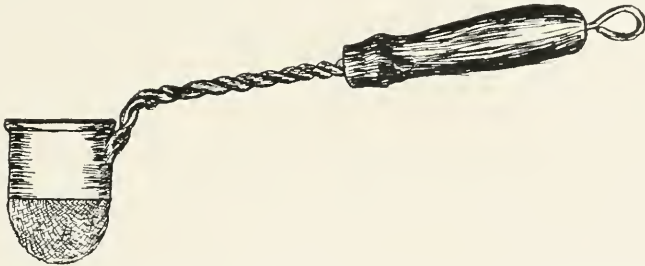
Zoölogical Laboratory, Columbia University.



### A New Section Lifter.

I have recently been doing a large amount of work on the anatomy of the stems of plants and have been using with good results a method for handling the tissues which does away with the long paraffin method. I give it here hoping that it may be interesting to some of the readers of the JOURNAL.

The block of tissue is passed through a killing fluid in the usual manner, absolute alcohol was used in this case. After the tissue has hardened in 96 per cent. alcohol for a few days it was cut in sections with the aid of a common hand microtome and section razor, the bit of tissue being placed between pieces of elder pith. In this way I was able to get sections 10 microns in thickness across the entire block. These sections were then taken with a brush from the razor and placed in a section lifter as shown in the drawing. This lifter is one



inch across the top and has for a bottom a piece of very fine gauze. It was set in a dish of absolute alcohol and the sections floated from the brush into the liquid which came up through the bottom. In this manner by simply setting the lifter in the fluid the sections could be passed through as many fluids as need be and finally floated out of it into a small dish of xylol or any other clearing agent. Then with the aid of a common section lifter they were placed on the slide and mounted in balsam. The same fluids were used as in the regular paraffin method of staining on the slide, but this method is much more rapid and I have had no trouble with either shrinking or clouding.

If care is used in cutting the sections so as not to crush the tissue and it is not allowed to dry out, rather soft tissue can be handled in this manner. I have used it with good results in making slides of the ovaries of some plants.

University of Vermont.

S. C. HOOD.

## The Technique of Biological Projection and Anesthesia of Animals.

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### XXI. DIRECTIONS FOR DEMONSTRATING BY PROJECTION THREE IMPORTANT PHENOMENA OF THE CIRCULATORY SYSTEM.—Concluded.

#### *A. The circulation of blood in the tail of a fish:*

This instructive experiment is best performed with small goldfish or silverfish measuring from two and one-half to three inches in length, as they require smaller and lighter cells than larger specimens. The fish is first anesthetized in chlorotone solution (see article XII of this series, March, 1903), and is then placed in a suitable cell (Fig. 9, No. 14, or one similar to No. 16, but larger), filled with the anesthetizing solution. It is not necessary that the cell should be large enough to contain the entire fish, but the tail-fin must be immersed in liquid during projection to prevent its being burned, and should be spread out in contact with the front side of the cell so as to be within reach of objectives of medium power. The head of the fish may be held up on one end of the cell by spreading the pectoral fins across the top of the cell, or in any other convenient manner. Low power objectives should be used. The special advantage for the study of the circulation afforded by the tail-fin of a fish is seen when the arterioles and venules near the capillaries are observed. These veins and arteries are seen to be parallel to each other and to the fin-rays and the flow of blood from an artery through the capillaries into a vein is more easily traced than in tadpoles or frogs. It will be noticed that the arteries lie close to the fin-rays. The repeated divisions of the fin-rays and their articulations are also points of interest worth noting.

#### *The circulation of blood in the web of a frog's foot:*

Examine the webs in the feet of the frogs which are available for the experiment and select one with a thin and light colored web. Anesthetize the frog as directed in article XIV (May, 1903). There are only two points in the demonstration that are likely to be difficult. First, the frog's web must be fully spread, and, second, the web must be immersed in water during projection. To spread the web take a piece of annealed copper wire about one-thirty-second of an inch in diameter and bend it into the form of a wide hairpin. Bend a half inch at each end of the wire back upon itself so as to form a narrow loop. Hold this spreader against the back of the frog's leg, slip the extremity of one toe into one loop and press the loop together tightly enough to hold the toe securely, and repeat the operation with another toe, either the next one or the second from the first. Now carefully bend the spreader so as to fully spread the web, being careful not to tear it. Keep the web moist while preparing it, and wrap the entire frog, except the foot bearing the spreader, in a moist cloth. The web is now ready to be immersed in water. The best cell to use is one having an opening at the top about one inch by one-half inch in area (Fig. 9, No. 17). Attach the cell to an adjustable frog plate or to a thin board having a hole one inch in diameter cut near one end. A strip of surgeon's rubber adhesive plaster will be found very

convenient for this, as for many other uses in the laboratory. Fill the cell with clear water, slip the frog's foot and the spreader into it and fasten the frog to the board by two or three thumb-tacks through folds of the cloth, or with twine. Use any objective having a sufficiently long working distance to reach the web. To check the rate of flow of the blood apply gentle pressure on the frog's thigh with a round pencil or penholder. To reverse the flow in the capillaries roll the pencil toward the foot while continuing the pressure.

*B. Directions for demonstrating the pulsation of the auricles and ventricles of the heart of a fresh-water clam :*

On account of the great variation in the thickness and convexity of the shells of different species it is best to select a specimen with thin valves and short lateral measurement. Thin valves are more easily ground off and the flatter the valve the thinner the cell required to hold the clam during projection. Having dissected a specimen of the species to be used in projection and determined the position of the heart and pericardial cavity with reference to the external markings on the valves, grind both of the valves thin—almost to the surface of the mantle—over an area larger than the entire pericardial cavity. Use an ordinary grindstone with plenty of water. Grind off too much rather than too little, but do not destroy the shell along the hinge line. Place the clam in a dissecting tray and cover with clear water. Break away and pick off the thin film of shell remaining over the pericardial cavity. Strong forceps and small bone-cutters are useful in this operation. Pick up the mantle lobe over the anterior end of the pericardial cavity with fine forceps and cut it away with scissors. The pulsating auricles are apt to get between the points of the scissors and be cut open so that the blood escapes and the auricle collapses. When the shell and mantle have been removed from both right and left sides of the pericardial cavity there is seen to be an opening through the clam just in front of the auricles and below the ventricle, and these organs expand into this space at each diastole. If the surrounding tissues interfere with the view of the pulsating organ, threads may be passed through the opening and brought around the shell and tied so as to keep the pericardial space clear for the passage of light in projecting. The specimen should now be kept entirely submerged in water during all subsequent manipulations, or the delicate auricles, deprived of the support of the pericardial liquid, may be destroyed.

The cell to be used in mounting the clam for projection depends upon the type of your projection apparatus. If it is fitted with a vertical reflector the clam may be placed in a tray of water without any cover. This, at first thought, may seem to be the easiest method, but it has this drawback that any vibration of the apparatus causes waves which seriously interfere with the perfection of the picture on the screen. The other method is to mount the clam in a large cell with an open top. The lens used in projecting the pulsating heart is the same as is used for lantern slides, unless the clam is a small one and the cell containing it can be placed on the stage of the projection microscope and a low power objective used.

A very instructive addition to this demonstration consists in a study of the effects of heat and cold on the rate of pulsation of the heart of this typical cold-

blooded animal. Having counted the number of pulsations per minute while the clam is in water at ordinary room temperature, cool the water in the cell to 45° or 50° F. by adding ice water, and again count the pulsations. Next add warm water enough to raise the temperature of the clam to 90° or 100° F. and note the increased rate of pulsation.

Clams prepared in this way may be kept alive in a tank of fresh clear water for some days, and used for several demonstrations by projection as well as for study with the eye and hand lens.

*C. Valvular action in the heart of a live animal and the movement of blood through the heart:*

This is an optical demonstration of one of the most completely hidden phenomena of the circulatory system. Nearly all the species of animals which have well developed cardiac valves also have hearts and surrounding tissues so opaque

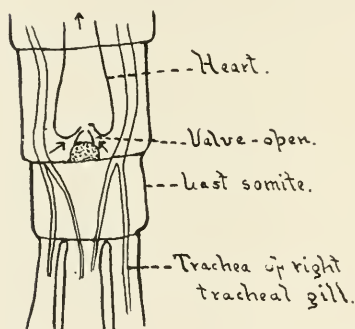


FIG. 11.—Diagrammatic sketch of the terminal somites of the abdomen of a nymph of Dragon-fly (*Agrion*) showing the position of the heart and its valves as seen in optical section.

as to prevent observation of their valves in action during life. The nymphs of certain species of Dragon-fly of the genus *Agrion*, illustrated in an earlier article in this series (Figs. 7 and 8, March, 1903) and described in the succeeding issue (April, 1903) are best adapted to this demonstration, but *Daphnia pulex* may also be used. It is probable that other animals will be found which may be used in this demonstration, and notice of such discoveries will be appreciated by the writer.

In selecting a nymph choose one with as little color as possible in the terminal segments of the abdomen, as seen under a hand magnifier or dissecting microscope. To anesthetize the nymph place it in one per cent.

chlorotone solution in a watch-glass. Fill a cell of suitable size and shape (Fig. 9, Nos. 5, 6. or 7), with the same solution and place the specimen in it with its dorsal side up, and cover with a selected glass. Place the specimen on a compound microscope and, using a one-half or three-fourths inch objective, focus into the last segment but one of the animal's abdomen. Adjust the mirror for a strong light and use a diaphragm of small aperture. The heart is thin walled, is widest near its posterior end, and narrows gradually anteriorly. When seen in optical section from the dorsal side, the walls and valves appear as in the diagrammatic sketch, Fig. 11. The blood enters the heart at its posterior end through two valved openings, one on each side near the central line of the organ. During diastole the anterior and posterior sides of the open valve are parallel, but during systole they are pressed closely together, and the rate of pulsation is so slow that their movements are easily followed. One must guard against being deceived by a vigorous and somewhat irregular muscular action, which moves the tracheal trunks situated on each side of and close to the heart, and is, apparently, concerned with the operation of the rectal tracheal gill. Streams of blood carrying many



corpuscles may be seen surging toward the heart, entering it during diastole, pausing an instant, and then rushing into the dorsal blood vessel during systole. Having found the heart and its valves with the compound microscope, transfer the specimen to the projection microscope and use as low a power as will give the necessary magnification on the screen. The light must be very brilliant on account of the partial opacity of the animal.

*Daphnia pulex* may be studied in essentially the same manner as the nymph of Dragon-fly. Anesthetize it in a mixture of one per cent. solution of chloretone one or two parts, water five parts. Mount in a hollow-ground cell (Fig. 9, No. 3). Its heart is somewhat lens-shaped with the valves on the dorsal side near the lateral margins. The valves are not as distinct as in the nymph of *Agrion*, and the normal rate of pulsation of the heart is extremely rapid. Repeated attempts to count it accurately have failed, but give counts ranging from 250 to 300 per minute.

In concluding this series of articles it is a pleasure to note that the growing demand for better projection apparatus is being met by manufacturers, and that the continued use of chloretone in the study of live animals is resulting in the attainment of excellent, and in some cases unexpectedly delicate and interesting results. It is the intention of the author to combine with this series other articles of interest and value and publish the whole in the form of a complete manual of biological projection. Correspondence with users of these or other methods of projection and anesthesia is desired.

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## Industrial Microscopy.

### III.

#### APPARATUS—Continued.

The best light to use for microscopical work is that from a northern sky. Direct sunlight cannot be used. The amount and character of the light entering the objective is a very important factor in obtaining good results. Hence the value of the substage condenser and diaphragm. Colored objects or those that have been artificially stained can be examined with a larger diaphragm opening than nearly clear objects, such as starches, natural fibers and the like. A little experimentation will soon teach the importance of this feature of manipulation and also the most favorable conditions.

The lenses must be kept clean. A clean old linen handkerchief or, better still, Japanese lens paper obtainable from dealers in microscopes should be used. Simply breathing on the lens to cover it with a dew and then gently wiping it with the linen or paper will ordinarily be found sufficient to clean them. Lenses should never be rubbed with the fingers. Lenses not cleaned by this method should be wiped with the paper or linen dampened in alcohol, ether or xylol according as may be found necessary, but excess of these liquids should be avoided since part of them are solvents for the materials by which some lenses

are cemented in the mounts. Microscopes should not be kept exposed in rooms where acid fumes are much liberated.

A few accessories for use with the microscope should be mentioned at this time, since in some kinds of investigations they are very important.

*The Polarizing Apparatus.* This consists essentially of two Nicol's prisms, one of which is placed below and the other above the object. The one below the specimen is called the polarizer and serves to polarize the light before it reaches the object. It is usually mounted in some form of collar so that it can be supported in the substage apparatus between the mirror and condenser.

The Nicol used above the object is called the analyzer. It is usually placed in one of three different positions, viz.: (1) it is fitted to set as a cap on top of the eyepiece mount; (2) it is placed in a special form of eyepiece between the two combinations of lenses, or (3) it is supported in some way in the barrel of the microscope between the objective and ocular. The last mentioned position is the common arrangement for the petrographical type of instrument.

Polarized light is especially useful in the examination of certain crystals, fibers and starches. In using the polarizing apparatus the substage diaphragm is opened wider than in ordinary light, since in the process of polarizing the light about half of the luminous effect is lost. The object to be examined is put in place and brought into view with the polarizer in position. The analyzer is then put in place and rotated around the vertical axis until the point is reached where the field appears darkest. In this condition, objects which are doubly refractive display colors to a greater or less degree. The intensity and shade of color change with changes in the relation of the object to the planes of polarization.

In order to increase the effect produced by substances that are only slightly doubly refractive a selenite disk should be employed. The disk giving red of the first order is the most useful for this purpose. It can usually be secured from the maker of the instrument, to fit within the mount of the polarizer. It produces a red field in the microscope in which doubly refractive objects appear either in some shade of blue or purple when in one position and in shades of orange or yellow when rotated through  $90^\circ$ . The contrast thus produced not infrequently constitutes an important factor in determining the nature of objects.

*The eyepiece micrometer* is a fine micrometer scale engraved upon a circular plate of glass which can be placed upon the diaphragm of the eyepiece by unscrewing the upper lens mount or else by means of the divisible eyepiece tube furnished with part of the compensating series. It is used in making microscopical measurements. The value of each of the scale divisions will depend upon the objective, ocular and tube length; and hence must be determined for each combination used. In determining this value, which we may call the *micrometer modulus*, and ever after when using it, the tube length must of course be that for which the objectives are calculated. This length is most frequently measured from the upper end of the draw tube where the ocular is inserted to the lower end of the barrel where the objective is screwed on.

To determine the modulus accurately a stage micrometer is required having a scale marked in divisions of .01 mm. (or else in fractions of an inch). If the

moduli of the various combinations are once determined and recorded for reference the stage micrometer will not be needed thereafter except for verification.

The mode of procedure is as follows: Place the eyepiece micrometer in place on the diaphragm of the ocular. The stage micrometer is also placed in position upon the stage of the microscope as an ordinary slide and the instrument focused upon it until the scale is in distinct view. The two scales are then seen simultaneously, and one superimposed upon the other.

For the sake of illustration suppose 20 of the divisions on the stage micrometer are found to coincide with 12 of those on the eyepiece micrometer. If the former is ruled in hundredths of a millimeter (10 micromillimeters) then for this particular combination of objective and ocular each scale division of the eyepiece micrometer equals .20 mm. divided by 12, which gives .0166 mm.; or as commonly expressed in microscopic work  $16.6 \mu$ . The Greek letter  $\mu$ , called *mu*, is the symbol used for one micromillimeter or micron (.001 mm.).

In making measurements with this combination thereafter the object to be measured is placed upon a slide upon the stage of the microscope as usual and the size determined in units of the divisions of the eyepiece micrometer. To reduce this reading to microns it must be multiplied by the micrometer modulus, which in the above supposed case was found to be 16.6.

Another method of making microscopic measurements is described by Ives<sup>1</sup> which is ingenious as well as novel. In this method the image of a jeweller's saw or some similar object placed in direct line with the source of light is brought into the focal plane of the objective by focusing the substage condenser and using the plane mirror. This method requires the use of a stage micrometer to obtain the micrometer modulus, but removes the necessity of the eyepiece micrometer. Though not as convenient as the method first described, good results can nevertheless be obtained if proper precautions are taken to always have the distances uniform.

For more complete directions concerning the use of the microscope and its accessories many books are to be had. Among these might be mentioned the following: "The Microscope," by Gage; "Manipulation of the Microscope," by Bausch; and "Practical Methods in Microscopy," by Clark.

Bureau of Chemistry, U. S. Dept. of Agriculture.

BURTON J. HOWARD.

## To Clean Homogeneous Immersion Objectives.

On page 2298 of the JOURNAL Mr. C. M. Clark mentions "the water" to replace benzene, xylene, etc., in cleaning homogeneous immersion objectives. I can confirm the statement of Mr. Clark entirely while adding to it a modification.

After having used an homogeneous immersion objective, I first clean the objective and the slide with a piece of old dry linen of fine texture, then I moisten an end of the linen with a little saliva and after having gently rubbed the objective front therewith I ascertain if the cleaning is perfect, using a magnifier in the examination.

Thanks to the slight quantity of soda contained in the saliva the cleaning is perfect and practically instantaneous.

I have used homogeneous immersion objectives since 1878, when they were introduced, and never, either for those used with cedar oil or for those with glycerine immersion (Tolles and Spencer) or for those of NA 1.60 with monobromonaphthalin, have I employed any other method of cleaning. The saliva cleanses so well that the front of my objectives are still as clear as when new.

Director of Botanical Garden of Antwerp (Belgium).

DR. HENRI VAN HEWICK.

<sup>1</sup> Ives, "Making Measurements in the Microscope," Journal Franklin Institute, July, 1902, p. 73.

## The Museum.

### XIII.

#### ADMINISTRATION.

The question of administration in museums only approaches serious proportions when the museum itself has important dimensions. The administration of many local and small museums is necessarily simple. One curator may have entire control of everything, and where on a slightly more extended scale the museum staff exceeds one, or equals five, problems of installation, labels, investigation, accessories, etc., are readily determined by conference and mutual suggestion. And yet even in so circumscribed an area of authority, autonomy is desirable to a certain degree.

It is evident at the outset that a museum has two aspects, its outer and inner. Its relations to the public, its own trustees, members or patrons, the city or town where it is placed, and from which it frequently draws a very substantial portion of its maintenance, are its external aspect. Its particular theory and practice within its own walls, and in the management, equipment, and schemes of exhibition in its separate departments, the purchase or exchange of specimens, duties and functions of its curators, plans of research or exploration, are its internal aspect. In any large museum therefore it is essential that the line of juncture between these diverse attitudes be expressed in a director of such nature and acquirements as to bring him in sympathy with scientific work, and also enable him to meet the questions of administration, this latter office involving the estimate of expenses, survey and criticism of building plans, and projects, regulations and rules for the public, assistants, cleaners, guards, engineers, and means and ways of enlarging the usefulness and popularity of the institution. The director also acts as an interpreter of the needs and scientific methods of the museum to the trustees and patrons.

But with this said, the emphatic declaration remains that the curator in a museum department should retain his autonomy, and the development and installation, the method and aims, the particular theories, and their exemplification, of presentation and popular instruction involved in his arrangement and disposition of the material at his command should be unmolested. In this way individuality can be given to a department, and while of course the contrasted character of their contents gives each taxinomic section of a museum a real isolation from the rest, the plans of labels, the scheme and order of the explanations, the various designs used by a curator for embellishing a collection as well as of making it a means of education to the public impart a special character quite different from the character derived from the objects themselves.

Perhaps complete laxity in the matter of supervision is to be deplored, but a very free use of his own power of initiative should be conceded to a curator, and its restriction only incurred when his methods appear extravagant or illusory or childish. Incompetency can only be rectified by suspension.



In the administration of a museum the interior aspect embraces, first, the control and guidance of its mechanical assistants, the engineer, janitor, etc., and secondly, the stimulation of the scientific departments, by meeting their needs, supplying their demands and recording their advances. In the former case it is well to adopt the policy of personal responsibility. A janitor or custodian is to keep the halls, windows, cases, etc., clean. He may also have charge of the receipt and despatch of packages, freight, etc., keeping a registry of the same; he must watch the character and deportment of the men under him, and might be expected to make a general report upon their duties and services. For all this he should be made personally responsible to the director.

The engineer attends to lighting, heating, plumbing, and, if such talent can be secured in the same man, inspection of the physical condition of the build-



FIG. 95.—British Museum; looking through gallery.

ings, whether they need repair for leaks, flooring, for renewed windows, roofing, etc., etc., and he also should report from time to time on the condition, needs, and the inventory of the apparatus in his charge. Much of this duty can be divided with a boss carpenter, who shall keep watch upon the deterioration of the building, and avert or correct it by requisition upon the director.

The relation of the executive to the scientific departments has been touched upon, but a system of considerable interest devised by Prof. H. C. Bumpus, whereby a record of their expenditures is accurately kept, merits explanation.

A book of blank requisition orders is furnished to each department, and the separate sheets in such a book are numbered, and stamped with the particular title of the department using them, as geology, invertebrate palæontology, verte-

brate palæontology, etc., etc. All the requirements of the departments are thus kept strictly tabulated, and these requisitions posted in a ledger under an account drawn up for each department furnishes an absolutely accurate registry of all expenses for the year, the cost of the requisition being ascertained and recorded in every instance. It brings also under the inspection of the director the character and extent of the requisition, and it involves his judgment and approval of every item of expense.

Many museums are supposed to be governed by a board of trustees who frequently furnish large amounts of money for the maintenance and expansion of the museum, or who act as intermediates between a city, or other public body, and the museum, receiving from the former, under certain stipulations, the necessary sums of money for its support. In many instances, as is particularly true of British museums, the government provides the same amount of money which is secured from private initiative and generosity. Now the director through the trustees, or the president representing the same body, becomes responsible for the expenditure of the public money, and a report or demonstration becomes imperative, showing the use and results of the expenditure of the public funds. Whether such a report is made to the trustees or to the government the intermediary office of the director is apparent. Through him the reconciliation is effected between the demands of the benefactors and the efforts of the beneficiary. A show of reason is given for the continued support, and the analysis or apology enumerates the stages of advancement, their features and their usefulness. It unfailingly is eloquent upon the great public advantage of the museum.

In administration is implied complete protection of property and the prevention of extravagance, prodigality and misuse of funds.

The curator represents the indispensable control which should give each department of a museum its peculiar expression, and his duties involve an appreciation of the popular and scientific features of installation. There can be no doubt as to the diverse aspects of a curatorship, and when along with the invention and art which is involved in giving the collections under his charge an attractive appearance, and the necessary systematic arrangement, and discharging his functions as an investigator, as well as taking the part of an instructor in talks, lectures, etc., the attainments needed are considerable. It is quite certain that these contrasted elements are not all readily combined, and it is a feasible proposition to give in large museums a divided expression to the departments, by the incorporation of the strictly scientific explorer with the duties of installation, exposition, correspondence and illustration.

In museums which depend upon public support, those which, in large cities, in consideration of their being made free to the public, receive a great municipal appropriation should industriously assist the public schools, making their contributions, in specimens, to the schools helpful in the courses of nature study. Such assistance reacts most favorably. It creates a public constituency which can be safely relied on in times of stress or persecution, or when more generous assistance from the city treasury seems essential.

There is one section of museum activity which seems pertinently alluded to under the heading of Administration. It is the interests and work of the taxi-

dermist, group-maker and modellers. The director of a museum is preferably, other things being equal, a man who especially has taste and facility in directing and estimating the value of these most essential features in museum installation. The taxidermist and his department is certainly a distinctive and autonomous interest in a museum, and yet, owing to the subserviency of its efforts to a number of departments, it has a divided realm of action, and might come to have a multiplied overseership. In this dilemma its independence is practically secured by bringing it under the supervision, control, and personal headship of the director, allowing, of course, to the taxidermist himself initiative concurrence and freedom of judgment.

#### SYSTEM AND EFFECT.

Quoting from a previous paper (*The Making of a Museum*), "the discussion of the installation of museums splits up at once into three groups, of equivalent importance perhaps, but of entirely divergent character. These three are Technique, System and Effect." Technique relates to or embraces mechanical adjustments, conveniences, receptacles, buildings, and the physical constants, or material. System relates to or embraces scientific sequence, illustration and information. Effect contains the whole subject of æsthetic presentation.

#### TECHNIQUE.

In the widest and apposite use of the term technique expresses the artisan phase of installation, reaching from illumination, which hints at the construction of the museum itself, to the best form of pins for suspension or insertion of specimens. It covers the multi-various details of *how* to exhibit an object, without bearing upon beauty of effect, or implications of science. It commends to the curator considerations of stability, of cleanliness, and of efficacy. Therefore it relates to the simple elements of construction, including in that term form and material.

Under form it discusses size, shape and arrangement of a hall of exhibition, or the *domiciliary*; size, shape and relations of cases, or the *loculus*; size, shape and relations of trays, supports, shelves, blocks, standards, pediments and all accessories of the same, or the *paraphernalia*.

Under material it discusses or compares the advantages of material entering into the domiciliary, loculus or paraphernalia, as wood, stone, iron, ivory, celluloid, paper, silk, plush, cotton, cork, paint, etc. To resume in a tabular form these distinctions we have :

Form	{ Domiciliary,
	{ Loculus,
	{ Paraphernalia.
Material—All useful fabrics.	

#### SYSTEM.

By system it is not implied that we are entitled to discuss classification of organisms or objects, whether minerals shall be arranged by formulæ or bases, plants by Gray's or Britton's manual, invertebrates by Cuvierian or Huxleyan methods, but there is implied by system, the discussion of and means and ways



of display that lead to certain intended results with reference to a mental impression on the spectator. Such systems are quickly comprehended under three heads, popular, philosophical, scientific, separated most naturally by the simple implication of the terms. The popular system informs the visitor what the objects are, bending on each a discriminating attention. The philosophical system develops the relations of objects to each other and to their environment; it may be teleological, it may be evolutionary, it may be simply spectacular. The scientific system tells of objects, their terminology, taxonomy, morphology, biology, and the varied aspects of living things, as deciphered by science.

How these results can best be attained, can be a legitimate consideration under installation.



FIG. 96.—Natural History Museum; London collection of shells.

The Popular system involves naturally an obvious use of striking, even sensational features, brilliant effects, simple phraseology, and profuse and intelligible comments and directions. It aims to lead the visitor with continuous interest from hall to hall, to punctuate his delight with distinct and delightful impressions, and to leave on his mind a sum of recognizable recollections. Its instructions are of the dictionary type, each object is clearly defined in and for itself, its relations are less accented and less evident. The popular system of the scientific museum is the system of the dime museum, greatly elevated, dignified, and replenished with culture, but still a practical appeal to the sensory centers of the spectator. The museum building in a popular system appeals to the eye, and has architectural beauty; its halls are large and form attractive vistas, prominent and beautiful objects are set off with strong features of color and mounting, and in collections the remarkable and beautiful are selected, and the obscure



and homely displaced. Thus in shells the large and showy only would be exhibited, the rest repressed; in minerals, the fine crystallizations, rare and dull species omitted; in birds, the magnificent and sumptuous, the plain and gray and dull neglected; in fossils, perfectly preserved and entire specimens or those in good relief, broken and shadowy things are consigned to drawers. The labeling would not be comprehensive or systematic but special. Each exhibit would be well explained, its relations ignored. You might learn much about the giant squid, you would not be shown its classification, congeners and physiology. You would see wonderful examples of quartz, you would scarcely realize its position amongst the other oxides. You would read of the habits of the bat, you would



FIG. 97.—Natural History Museum, London; bucks and antelopes.

not understand the homologies of its limbs. You might admire the size of a whale's skeleton, you would not realize its position amongst the mammalia. Of course no museum of natural history to-day defers entirely to a system so juvenile and fractional, although all museums are increasing their respect for its appreciation of *effect*, its evident intention to make the visitor stop and admire. The popular system is a sub-dominant note in the chord struck by the whole administrative faculty of a museum.

The Philosophical system aims at unfolding an idea. It is less concerned with a multitudinous display of species than with developing the regimen those species illustrate. This treatment is well illustrated in the main central hall of the British Museum of Natural History, where a series of cases present formative principles in animal life. Thus the group of pigeons, showing the varia-

tion of a species under domestication as the derived varieties from the Wild Rock Dove (*Columba livia*). Again, the modifications of the Jungle Fowl of India, where extreme changes may be noted, as in the Japanese longtailed fowls, and the fowls of the woods of the Fiji Islands. Also the group of Ruff and Reeves, illustrating variation, according to sex and season. Demonstration of color adaptation, Protective Mimicry, Albinism, Melanism, etc., all present the Philosophical system; while the same, carried still further, beyond the limits of mere teleological considerations, converts the museum into an embodiment of an evolutionary thesis.

In this way, from the inorganic through the first phases of organic life to its crowning development in man with all the related phases of ascending civiliza-



FIG. 98.—South Kensington Museum; north court.

tion, what a transcendent picture of life the museum may become! It is perhaps realized nowhere to-day because the opportunity and the governing mind are not anywhere associated. The Philosophical system in anthropology and ethnology affords a field of more than surpassing dimensions.

The Philosophical system has but a slender regard for systematists, and exults rather in revealing relations, sequences and operations in nature, homologies and analogies, influence of environment, problems of philogeny and those aspects of animal life which elucidate the principles of organic variation. It can, of course, be made most attractive, and has a more popular character than the Scientific system. Its instructions are for the most part quite readily apprehended, or can be made so, and in the larger subjects its demonstrations admit of a considerable pictorial effectiveness.

## THE SCIENTIFIC SYSTEM.

The Scientific system aims at an exhaustive display of species arranged in botany and zoölogy, according to their biological affinities, and in palæontology according to biology and position, while in inorganic life it illustrates the entire range of mineral science. This is the more common, the more generally insisted upon, form of museum installation. It is well understood; cases filled with examples of all the known or obtainable specimens of species. At its best, when it takes on, more and more, a philosophical expression, the Scientific system uses diagrams, photographs and maps, to illustrate anatomy, habits and distribution, and it does not hesitate to involve popular features in its work, describing special things with clearness and interest. Indeed an enlightened scientific treatment tries to alleviate the dryness of terminology with popular and



FIG. 99.—South Kensington Museum; south court.

informing features. The curator who thinks his science is invalidated by entertaining instruction to the public is certainly deceived.

The cosmopolitan museum will make use of all these systems, building up from the scientific, as a basic method, and introducing the philosophical at all necessary points, while the popular treatment would prevail like a dominant influence over each.

## EFFECT.

Effect is quickly understood. The æsthetic quality of a display is gauged upon inspection. And such effects are numerous: tasteful, impressive, sensational, sumptuous, plain; but referring always to visual impressions affecting our sense of beauty, propriety, clearness, etc.

Impressions made upon the eye are of the utmost importance in museum

installation. There may be some atrophied and stagnant temperaments to whom a beautiful or tasteful or impressive installation seems at war with the terribly serious considerations of science, but a very little attention to the facts of the case would entirely relieve them of these fears. Because a specimen looks well, it is no less the same specimen than when it looks poorly, and all cultivated instinct aims to achieve, in making it look well, is to make it more easily seen, make it more conspicuous. There certainly is no desire, in those who strive for effect, to surround objects with decorations which defeat their own purpose, and bring more attention to the embellishment than to the object. The most refined appreciation of effect sees that the different departments of a museum may need differing treatments and that severity of arrangement better accords with a display of building stones or ores than ornament, while the lavish beauty of birds may demand foils and reliefs to their beauty to even make it more apparent.

In effect, arrangement, and color contrasts count for something. Proper spacing, selection of material, and backgrounds of good fortifying colors make notable improvements in the appearance of the specimens. Besides the painting of the cases and shelves, the use of cloth, plush, and paper can be utilized. It is certainly undesirable to attempt harlequin effects, and usually a few selected colors meet every requirement.

In the figures here used some illustration is given of the methods adopted in museums for exhibition, and they cannot in all cases be regarded as eminently successful, though the north court of the South Kensington museum is an attractive example of varied and entertaining installation and also is an instance of top-lighting. The objects are perhaps a little too crowded, but there is considerable art displayed in their contrasted juxtaposition and mounting.

The arc lights in use here for illumination do not seem at all as attractive and appropriate as grouped incandescent lamps. The south court of the same museum, also shown, would be improved if the alignment of the cases was not so monotonously similar. In the shell hall of the National History museum, London, the walled in effect is disagreeable and repressive, and could be dissipated by lining the walls with shallow cases carrying exhibits. Such illustrations might be endlessly supplied. It is fortunate under all circumstances that individual preferences vary and the exercise of individual invention or design produces diverse and manifold results.

The museum has become part of the educational life of our cities, and must increasingly solicit public consideration and public support.

American Museum of Natural History.

L. P. GRATACAP.



## Laboratory Outlines for the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

### SUBKINGDOM, ANGIOSPERMAE.

A number of forms to represent the general evolution of the flower in monocotyls and dicotyls.

LXXVI. *Sagittaria latifolia* Willd. Arrow-head.

Class, Monocotyledones. Order, Niadales. Family, Alismaceæ.

The wide-leaved arrow-head grows in moist ground on the margins of ponds, creeks and canals. If fresh material is not available, good herbarium specimens may be used. Flowers and other parts may also be preserved in alcohol.

*Sporophyte.*

1. Sketch and describe the entire plant, noting the character of the leaves, stem, roots, and inflorescence.

2. Sketch the staminate flower, showing sepals, petals and stamens. How many parts in each set?

3. Sketch the carpellate flower and describe the parts present. What parts of the two flowers are cyclic and what parts spiral in arrangement? Is this sporophyte monœcious or diœcious?

4. Under dissecting microscope draw a sepal, a petal, a stamen (microsporophyll) and a carpel (megasporophyll).

5. Cut cross sections of a young stamen, mount, and draw under low power. How many microsporangia? Note that the stamen is made up of anther and filament.

6. Cut off one side of a carpel so as to expose the ovule (megasporangium). Draw under low power, showing the stigma, short style, and ovulary. Note that the stigma is a new organ not present in any of the forms previously studied. Why is the stigma necessary to this carpel?

LXXVII. *Ranunculus abortivus* L. Crowfoot.

Class, Dicotyledones. Order, Ranales. Family, Ranunculaceæ.

This plant is common in April and May along brooks, on hillsides, in meadows, and along roads.

*Sporophyte.*

1. Sketch the entire plant, showing the various organs.

2. Sketch the flower and describe the condition of the four sets of floral organs. Note that the flower is bisporangiate. Compare with the cone of *Selaginella*.

3. Draw a sepal, a petal, a stamen, and a carpel under dissecting microscope.

LXXVIII. *Alisma plantago* L. Water Plantain.

Class, Monocotyledones. Order, Naiadales. Family, Alismaceæ.

The water plantain is common in wet and muddy places, on the margin of ponds and creeks. Herbarium specimens and preserved material may be used.

*Sporophyte.*

1. Sketch a leaf and a part of the inflorescence.
2. Sketch a flower showing the four sets of floral organs—calyx, corolla, andrœcium and gyncœcium. How many sepals, petals, stamens and carpels? Are the parts spiral or cyclic? Free or united? Is the flower monosporangiate or bisporangiate? Note that the flower is hypogynous. What advance does this flower show over that of *Sagittaria* or *Ranunculus*?

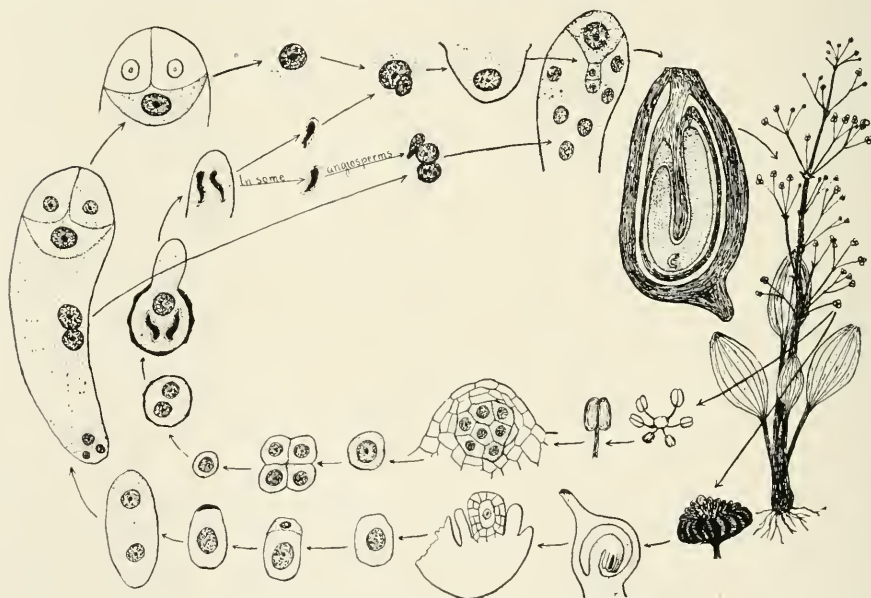


FIG. 14.—Diagram of life cycle of Angiosperm (*Alisma*).

3. Make a diagram of the flower. See Fig. 15 a.
4. Cut cross sections of the stamens and draw under low power. How many microsporangia (pollensacs)? Cut open the ovulary and dissect out the ovule (megasporangium). Draw.
5. From prepared slides draw a microsporocyte and a microspore, showing the nucleus, cytoplasm and wall.
6. From prepared slide draw a young ovule, showing the funiculus, the integuments, the megasporangium proper (nucellus), and the single megaspore. Note the absence of a wall around the megaspore. Why not present?

*Gametophytes.*

7. From prepared slide draw a male gametophyte (pollengrain), showing the tube nucleus and the two elongated sperm cells.

8. From prepared slide draw an eight-celled female gametophyte (embryo sac), showing the three antipodal cells, the two polar nuclei, the oosphere, and the two synergids.

9. From prepared slide draw a mature, seven-celled female gametophyte, showing the conjugation of the polar cells to form the definitive cell.

10. From prepared slide draw an embryosac with endosperm cells, which have come from the division of the definitive cell, and with young embryo, consisting of the embryo proper, the suspensor cells and the large, vesicular, basal, suspensor cell. Note that the conjugation of the polar cells and the subsequent development of the endosperm are entirely new phenomena, nothing similar occurring in plants below the angiosperms. In many angiosperms the second sperm cell from the pollentube comes down and unites with the definitive cell producing a triple fusion.

11. Carefully remove a mature embryo from the seed and sketch under low power, showing the single cotyledon, the lateral plumule and the radicle.

12. Note that in this plant the seed remains in the ovulary. Make a diagram showing position of the carpel wall, the integuments of the ovule, the endosperm, and the embryo.

13. Sketch a young seedling.

14. Make a diagram in the notes showing the general life cycle of an angiosperm. See Fig. 14.

#### LXXIX. *Sedum acre* L. Wall-pepper.

Class, Dicotyledones. Order, Rosales. Family, Crassulaceæ.

Many of the sedums grow well in greenhouses and in window gardens. They usually bloom abundantly in the spring and the above or any other species will be found suitable.

1. Make a careful drawing of the flower and describe the character of the different parts.

2. Make drawings of the calyx, the corolla, the andræcium and the gynæcium.

3. Answer the following questions correctly :

Is the flower hypogynous, perigynous, or epigynous ?

Is it tetracyclic or pentacyclic ?

Are the circles or whorls trimerous, tetramerous or pentamerous ?

Are the organs of any whorl or set united or partly united ?

Is the flower isocarpic or anisocarpic ?

Is it actinomorphic, isobilateral, zygomorphic, or nonsymmetrical.

4. Make two diagrams showing the true condition of the flower as learned above. See Fig. 15 a. and b.

#### LXXX. *Trillium grandiflorum* (Mx.) Salisb. Large-flowered Wake-robin.

Class, Monocotyledones. Order, Liliales. Family, Liliaceæ.

The large-flowered wake-robin grows in rich woods and blooms in April and May.

1. Make a sketch of the entire plant, showing the flower, leaves, and short

tuberous rhizome with contractile roots below. How deep was the rhizome under ground? Describe how it descends into the earth. This plant is a geophilous, herbaceous perennial. What are the advantages of the geophilous habit?

2. Cut a cross section of the compound ovulary, mount, and draw under low power, showing the cavities with ovules.

3. Describe the condition of the flower according to the questions asked under *Sedum acre*. Make a diagram of the flower.

LXXXI. *Cypripedium hirsutum* Mill. Large Yellow Lady's-slipper.

Class, Monocotyledones. Order, Orchidales. Family, Orchidaceæ.

This lady's-slipper grows in wet places and low woods, and blooms in May and June.

1. Sketch part of a plant, showing the flower and part of the leafy stem.

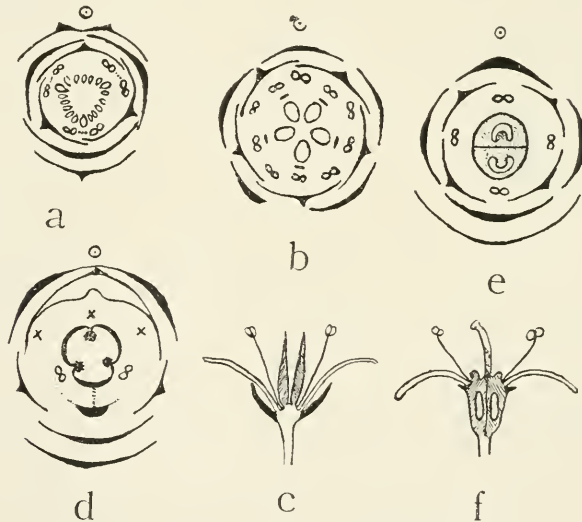


FIG. 15.—Diagram of flowers.

2. Cut cross sections of the ovulary, mount, and draw. How many carpels? Study the flower with the aid of the diagram, Fig. 15 d.

3. Copy the diagram in the notes and write a general description of the flower, noting especially that it is organized on the same plan as the *Trillium* flower, that some of the parts have disappeared, that it is epigynous and zygomorphic, that certain parts are united, and that it is highly specialized for insect pollination.

4. Why should this flower be placed higher than any of the monocotyls previously studied? Make a comparison of the flower of *Sagittaria*, *Alisma*, *Trillium* and *Cypripedium*.



LXXXII. *Catalpa speciosa* Warder.

Class, Dicotyledones. Order, Polemoniales. Family, Bignoniaceæ.

The *Catalpa* is cultivated extensively and blooms abundantly in May and June.

1. Study the large compound panicle and draw a single flower.
2. Describe the flower carefully, noting the condition of each floral set and whether the flower is hypogynous or epigynous, whether actinomorphic or zygomorphic. What adaptations for insect pollination? Note especially the rudimentary or vestigial stamens.
3. Cut cross sections of the ovulary, mount, and draw. How many carpels in the gynæcium?
4. Make two diagrams of the flower, showing transverse and longitudinal arrangement.

LXXXIII. *Cornus candidissima* Marsh. Panicked Dogwood.

Class, Dicotyledones. Order, Umbellales. Family, Cornaceæ.

This common shrub usually forms thickets, in forests and on hillsides. It blooms in June, producing an abundance of flowers.

1. Sketch the entire inflorescence and note the arrangement of the numerous small flowers.
2. Under dissecting microscope draw a single flower. How many cycles? How many stamens, petals and sepals? Note the minute size of the calyx.
3. Cut a cross section of the ovulary. Draw under low power. How many carpels? Note that the flower is epigynous.
4. Make a transverse and a longitudinal diagram of the flower. See Fig. 15 e. and f.

LXXXIV. *Ageratum conyzoides* L. Ageratum.

Class, Dicotyledones. Order, Asterales. Family, Compositæ.

*Ageratums* are annuals which bloom all summer and are much used for borders. The flowers may be had in the greenhouse at any time of the year. The plants will live and bloom for a long time.

1. Sketch one of the heads under dissecting microscope, showing the bracts of the involucre and the numerous small tubular flowers.
2. Under dissecting microscope draw a single flower. What is the condition of the pappus? What does the pappus represent?
3. Dissect the flower and draw the corolla, the andræcium and the gynæcium under dissecting microscope or low power. Describe the flower and its parts in detail.

LXXXV. *Chrysanthemum leucanthemum* L. Ox-eye Daisy.

Family, Compositæ.

This plant grows in fields and meadows and blooms in May and June.

1. Draw one of the heads, showing the bracts of the involucre, the ligulate or ray flowers and the tubular or disc flowers.
2. Under dissecting microscope draw a ray flower and a disc flower.

Describe each. What is the condition of the calyx? Why should the outer flowers develop as ray flowers rather than the inner ones? Note that the ray flower is zygomorphic.

LXXXVI. *Taraxacum taraxacum* (L.) Karst. Dandelion.

Family, Compositæ.

The dandelion blooms from early spring to late autumn, so plants may usually be obtained without difficulty.

1. Sketch an entire plant, showing root, short stem, rosette of leaves, and slender stems bearing heads of flowers. Note that the dandelion is geophilous. Why does it not grow up out of the ground? How do you account for the rosette habit?

2. Make a sketch of a single head. Note that all the flowers are ligulate.

3. Under dissecting microscope draw a single flower. Describe the papus, corolla, andræcium and gynæcium.

4. Draw some of the ripe fruit. Note adaptation for suspension in the air. Of what special advantage is this parachute arrangement? Note the action of the involucre while the fruit is ripening.

5. How many seeds in each dandelion fruit? i. e., how many for each flower? How many seeds are produced on an average-sized head? About how many heads of flowers are matured from a fair-sized dandelion plant in one season?

6. Suppose that you had one mature dandelion plant and that it produced seed normally for ten years and that each seed developed into a mature plant and began to reproduce at the normal rate at three years of age, how many off-spring would there be at the end of ten years?

7. The total land surface of the earth is about fifty-three millions of square miles. Supposing that it were possible to distribute the seed uniformly over the land surface and that all seeds had a suitable environment for sprouting and growing into mature plants, how many dandelion plants would there be for each square mile of land surface at the end of ten years?

8. Note.—The above problems will indicate to some extent the great possibilities of reproduction present in many plants. It will be remembered that each seed contains a little, dormant embryo; therefore, every seed that perishes means the destruction of a young plant. It is evident that a very large per cent. of young plants must perish each year, and that those which survive for any length of time must usually undergo a severe struggle for existence. In this struggle for life and place the fittest usually survive; i. e., those which are able to grow more vigorously and thus overshadow their weaker neighbors and those which are best able to adapt themselves to their environment.

#### APPENDIX TO ANGIOSPERMS.

LXXXVII. *Comparison of Carpels.*

1. If not previously studied, draw a carpel of *Cycas revoluta* L.

2. Draw a carpel of the Kentucky coffee-bean (*Gymnocladus dioica* (L.) Koch).

3. Carefully separate the ovularies of the carpels of an orange (*Citrus aurantium* L.) so that they will lie side by side in a row. Draw. Note that some of the divisions are smaller than the normal. There is a struggle for existence among the members of the gynæcium so that some are not fully developed.

4. Make a comparison of the three fruits studied above.

#### LXXXVIII. *Section of Sunflower Leaf.*

1. Cut cross sections of the lamina of a sunflower (*Helianthus annuus* L.) leaf preserved in alcohol, mount, and study under low and high power.

2. Draw and describe, showing the following tissues: upper epidermis with thick cuticle and multicellular hairs, palisade parenchyma, spongy parenchyma with large intercellular spaces, sections of vascular bundles, and lower epidermis with stomata and multicellular hairs.

3. How do you account for the palisade arrangement of the cells in the upper part of the leaf?

#### LXXXIX. *Leaf Variation.*

1. Obtain a series of fresh or pressed leaves of the red mulberry (*Morus rubra* L.) or of the giant ragweed (*Ambrosia trifida* L.) and make outline sketches of ten different forms.

#### XC. *Section of Winter Bud.*

1. From alcoholic material cut longitudinal sections of common lilac buds (*Syringa vulgaris* L.). Mount and sketch under low power. Note the flat apex with outer dermatogen and hypodermal meristematic tissue; and a little farther down the epidermis, cortex (periblem), procambium (formative tissue of vascular bundles), and the central pith.

2. Note the origin of the leaves, beginning at the apex, and also the origin of the lateral buds in the axils of the leaves. Make a sketch showing the entire upper part of the bud, with all the structures mentioned above.

#### XCI. *Monocotyl Stem.*

1. Cut cross sections of young corn stems preserved in alcohol, stain and mount; or use prepared slides. Sketch the entire section under dissecting microscope, showing epidermis, band of sclerenchyma, large pith or ground tissue, and the scattered vascular bundles.

2. Under high power draw one of the bundles. Note the large vessels situated in the xylem arranged like a letter V, the cavity in the tissue at the apex of the V, the bundle of phloem between and beyond the two large vessels, and the sheath of sclerenchyma about the bundle.

#### XCII. *Herbaceous Dicotyl Stem.*

##### (a) *Sunflower Stem.*

1. Cut cross sections of a young sunflower stem preserved in alcohol, mount, and stain; or study prepared slides.

2. Sketch the entire section under dissecting microscope, showing cortex, circle of vascular bundles, and large central pith.

3. Under low power draw part of a section showing the epidermis with epidermal hairs, the layer of colenchyma immediately below this, the parenchyma with resin passages, the vascular bundles with cambium layer, the medullary rays, and the central pith.

4. Under high power draw a single vascular bundle, selecting one of the narrow, oval type. Represent in order the external bundle of sclerenchyma, the phloem, the cambium, and the xylem usually in a double layer.

5. Notice the mechanical principles involved in the structure of the stem and the vascular bundle. Compare with a T railroad rail.

(b) *Pumpkin Stem.*

1. Cut cross and longitudinal sections of the stem of *Cucurbita pepo* L., stain and mount, or study prepared slides. Sketch the cross section under dissecting microscope. Note the epidermis, the cortex, the vascular bundles, the pith, and the large central cavity.

2. Under high power study the longitudinal sections and draw sieve-tubes showing the sieve-plates in the phloem.

3. In the xylem find and draw one of the large reticulate wood vessels; also a spiral wood vessel with a single spiral thickening and one with two spirals; also draw an annular wood vessel in which the thickenings are in the form of rings.

XCIII. *Dicotyl Woody Stem.*

1. Cut cross sections of linden twigs (*Tilia* sp.) preserved in alcohol. Take one very young branch, one a year old, one two years old, and one three years old. Mount and stain; or use prepared slides. Study under low and under high power.

2. Make a series of diagrams showing the primary structure and how the secondary structures are developed for the first three years.

3. Make a sketch of a cross section of a polished tree trunk of black walnut (*Juglans nigra* L.), showing these structures: pith, heartwood (duramen), sapwood (alburnum), annual rings with spring and fall wood, medullary rays, the stelar cambium and phloem (inner bark) separated by irregular strips or arcs of corky tissue from the outer bark. The outer bark has been developed from the cortex and phloem and modified by successive layers of cork cambiums (phellogen). These irregular strips of corky tissue can easily be seen in the outer bark with the naked eye.

4. Compare with the walnut a polished section of the trunk of a bur oak (*Quercus macrocarpa* Mx.). In this the medullary rays are much more prominent.

XCIV. *The Root.*

(a) *Section of Buckeye Root.*

1. Cut cross sections of one of the larger fleshy rootlets of *Aesculus* sp. (preserved in alcohol). Mount and draw under low power, representing the following structures: the four or more primary xylem bundles, four or more primary



phloem bundles alternating with the xylem, the beginning of the stelar cambium passing between the xylem and phloem, the endodermis or bundle sheath, and the broad cortex with a superficial layer of cells known as the piliferous layer.

2. Cut cross sections of a somewhat older root which has turned brown, mount, and sketch the entire section under low power. Represent the following structures: the central strand of xylem composed of wood vessels and smaller cells, the stelar cambium, the band of phloem consisting of several kinds of cells, the remains of the endodermis, and the cortex and piliferous layer turned brown.

(b) *Embryonic Root Tip.*

1. Carefully remove the hard parts around the base of the embryo in a grain of corn (*Zea mays* L.) and with a razor cut longitudinal sections of the radicle of the dormant embryo. The corn may be soaked in water for a while before cutting the sections, though this is not necessary.

2. Mount the central sections in water and sketch under low power. Note the following embryonic tissues: the outer scutellum, the root-sheath of a dark appearance inside of the scutellum, and the root tip inside of the root-sheath. The root tip is made up of the root-cap (of a light color), the dermatogen (a layer of large cells inside of the cap), the dark layer of periblem, the central light plerome, and the growing point at the tip of the plerome. Outside of the dermatogen, at the apex of the root is the formative tissue of the root-cap, known as the calyptragen. It will probably not be distinct enough in these sections to trace out, but its position should be noted.

(c) *Root Hairs.*

1. Sprout grains of corn on moist blotting paper in a box or under a bell-jar; after a few days the roots will be covered with root hairs. Sketch under dissecting microscope.

2. With a scalpel cut off some of the epidermis containing root hairs, mount and examine under high power. Draw and describe.

3. Under low power examine roots of young seedlings planted in soil and note the relation of the root hairs to the soil particles.

XCIV. *Lenticels.*

1. Examine and sketch the bark of a green and of a year-old elder stem (*Sambucus canadensis* L.) showing the surface covered with lenticels.

2. Cut cross sections of the bark, mount, and examine under low power. Sketch one of the lenticels. How and where do they originate?

XCVI. *Starch, Cellulose and Lignin.*

1. Cut a potato and scrape off some of the cells. Mount in water and study under high power. Draw some of the large starch grains present, showing the hilum and the stratified structure.

2. Place a drop of iodine solution beside the cover-glass and watch its effect on the starch. What is the color reaction?

3. Mount some wheat flower in water and treat with iodine. Note the blue colored starch and the yellow colored proteid material.

4. Mount a hair of common cotton (*Gossypium herbaceum* L.). It is made up of nearly pure cellulose except the small central cavity in which is a small amount of dry protoplasm. Draw. Treat with Schulze's solution (Chlor-zinc-iodine) and after a while note the color reaction. Care must be taken so as not to get any of this solution on the microscope as it is strongly acid. Schulze's solution can be obtained from the dealers.

5. Test a cross section of a sunflower stem (from alcoholic material) with Schulze's solution, examine, and note the cellulose reaction in walls of the cortical and pith cells.

6. Treat a section of a sunflower stem with phloroglucin, mount and study color reaction in the xylem bundle. Care must be taken in its use as it contains an acid.

7. Cut cross sections of a young twig of linden preserved in alcohol, treat with phloroglucin, mount, and note color reaction in the wood.

#### XCVII. *Crystals.*

The material for sectioning may be preserved in alcohol.

1. Cut cross sections of the rhizome of the large blue-flag (*Iris versicolor* L.), mount, and under high power draw the simple crystals present.

2. Cut sections of rhizome of the wild crane's bill (*Geranium maculatum* Tourn.), mount, and draw the large compound sphere-crystals.

3. Cut sections of the rhizome of the lily-of-the-valley (*Convallaria majalis* L.), mount, and draw the bundles of needle-shaped crystals, raphides.

4. Cut cross sections of the leaves of the India-rubber fig (*Ficus elastica* Roxb.), mount, and draw the large cystoliths which are amorphous masses of mineral substance suspended from a pedicel. The mineral substance of the cystolith is mainly calcium carbonate.

#### XCVIII. *Lipochrome.*

1. Cut thin sections of the rind of an orange, mount in water, and examine under high power. Draw a cell showing the chromoplasts.

2. Cut sections of the root of the common cultivated carrot (*Daucus carota* L.). Mount and draw a cell showing the chromoplasts.

3. Mount pieces of the yellow corolla of the squaw weed (*Senecio aureus* L.) or any other yellow flower, examine under high power and draw a cell with chromoplasts. Describe the cause of the yellow color in these tissues.

#### XCIX. *Anthocyan.*

1. Cut sections of the root of the red garden beet (*Beta vulgaris* L.), mount, and examine under high power. Note that the red coloring matter is in the cell sap.

2. Cut sections of any leaf with red color as the red leaved coleus (*Coleus blumei* Benth.), mount, and study the color under high power.

3. Cut off some of the epidermis of a red apple (*Malus malus* (L.) Britt.), mount, and study the cause of the color.

4. Mount part of a petal of a red greenhouse Pelargonium. Study the red coloring matter in the cells.

5. Mount part of a petal of a blue flower (like *Salvia pitcheri* Torr. or *Viola cucullata* Ait.) and study the nature of the color.

### C. *Solution of Anthocyan.*

1. Take a quantity of the corollas of *Maurandia barclaiana* Lindl. (a common greenhouse vine) or flower of *Tradescantia virginica* L., place them in a dish and after crushing them cover with a quantity of 95 per cent. alcohol. After a day or so pour off the alcohol into a bottle and preserve.

2. Take a test-tube about one-third full of the alcohol and add a few drops of aqua ammonia. Note color reaction. Neutralize with hydrochloric acid until the liquid is again clear. Continue to add acid drop by drop. What is the color?

3. Place some red pelargonium flowers directly into ammonia water. Note that they change to blue. Transfer to acid alcohol and note that they change back to red.

4. How do you account for the change of color in many flowers during the period of blooming and for the many varieties of color produced by cultivation as in the common morning-glory (*Ipomaea purpurea* (L.) Roth.)?

### CI. *Temperature Test with Anthocyan.*

1. Take two good thermometers which register alike, wrap the bulb of one in a red begonia leaf and the other in a green begonia leaf, put each in a tumbler and place for some time in direct sunlight. Note the difference in temperature. Place the tumblers with thermometers in diffuse light and note temperature again. Place them in a dark box and after a while read the temperature. Make a second test in the sunlight.

2. Describe one of the uses of anthocyan in roots, stems, leaves, flowers and fruits.

### CII. *Chlorophyll Solution.*

1. Take a quantity of green leaves, such as the blue grass or greenhouse pelargonium; place them in a porcelain mortar or other suitable dish; cover with 95 per cent. alcohol; and crush the leaves thoroughly. After the alcohol is colored a dark green filter into a bottle and keep in a dark place.

2. Take a small quantity in a test-tube and examine by looking through it toward the window. Note the deep green color produced by the transmitted light. Examine it by reflected light, by standing between the window and the tube, and observe that the color of the solution appears a deep dull red, something like blood.

3. Take a small quantity of the solution in two test-tubes, and place one in the sunlight and the other in a dark box. How long before the one in the sun-

light fades out? Compare it with the one in darkness. Thus it will be seen that sunlight when too intense will rapidly change the character of chlorophyll, although it is generally absolutely necessary for its development.

### CIII. *Latex.*

1. Take one of the large, red, deciduous stipules which cover the terminal bud of *Ficus elastica* at the time when it is becoming transparent, a few days before it is ready to fall. Examine immediately by holding the stipule with the inner side up on the stage of the microscope and examine with low and high power. Note the complex system of lactiferous ducts and the movement of the latex in them caused by its escape from the torn end of the stipule. At times the flow in the ducts appears to be very rapid.

2. Mount some of the latex and examine under high power. Note the spherical granules and draw. These are the rubber globules.

3. Why does the stipule become colored before it drops off from the bud?

### CIV. *Pollentubes in Artificial Cultures.*

1. From an opening anther take fresh pollen of Canna, Hyacinth, or Begonia and make cultures in the following solution:

a. Cane sugar	-	-	-	-	6 parts.
b. Gelatin	-	-	-	-	3 "
c. Tap water	-	-	-	-	91 "

Heat the mixture over a water bath till the gelatin is dissolved.

2. To a centimeter or two of this solution add an equal quantity of tap water and filter into a small covered dish. Put the pollen into the solution and also make hanging drop cultures, placing the slides into a moist chamber.

3. In 20 to 24 hours examine and draw several tubes representing successive stages of development. Note the rotation of the cytoplasm.

4. Treat with iodine solution and note the position of the nuclei.

Ohio State University.

JOHN H. SCHAFFNER.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Holden, R. J., and Harper, R. A. Nuclear divisions and nuclear fusion in *Colcosporium sonchi-arvensis*, Lev. Trans. Wisconsin Acad. of Sciences, Arts and Letters, 14: 63-82, pls. 1-2, 1903.

At certain stages in the life history of this rust, the cells contain two nuclei and at other stages but one nucleus.

The uredospore, and the cells of the mycelium to which it gives rise, contain two nuclei which divide by conjugate division, i. e., each nucleus contributes to the two daughter cells. The teleutospore produced from this mycelium is the last binucleated cell of the series. The two nuclei of the teleutospore fuse, after which the teleutospore at once germinates into a four-celled promycelium, each cell of which contains but a



single nucleus. Each of the four cells of the promycelium produces a uninucleated sporidium. The first division of the nucleus of the sporidium is not followed by cell division, but starting with the sporidium there is developed a mycelium of binucleated cells. In short, from teleutospore to sporidium the cells are uninucleated, and from sporidium to teleutospore, binucleated.

The two nuclei which fuse in the teleutospore maintain a separate existence throughout almost the entire life cycle of the rust, and there is some evidence that the chromosomes in the division of the fusion nucleus are collected into two groups, representing, possibly, the chromosomes of male and female nuclei. While there is no proper cell fusion, the union of nuclei more or less separated in origin is not out of harmony with our conceptions of sexual reproduction in other groups of plants.

C. J. C.

**Kohl, Dr. F. G.** Ueber die Organization und Physiologie der Cyanophyceenzelle und die mitotische Teilung ihres Kernes. 8vo. pp. 240, 10 pls. Gustav Fischer, Jena, Germany, 1903. Price, 20 marks.

The structure of the Cyanophyceæ and Bacteria has long been a subject of dispute, and the observations, interpretations, and theories have been conflict-

ing and contradictory. Some writers have described nuclei and chromatophores while others have denied the existence of such organs, and in regard to other structures only less difference of opinion has prevailed.

Dr. Kohl gives a critical review of the work of previous investigators and presents the results of his own extensive investigations, which deal chiefly with *Tolypothrix*, *Nostoc* and *Anabaena*. Without attempting to separate original views from confirmations and contradictions, the author's conclusions, as found in his summary and in the body of the work, are about as follows: The protoplast of the Cyanophyceæ does not differ essentially from that of other plant cells. It has a nucleus and peripheral cytoplasm containing chromatophores. There is always a single nucleus in a cell, and it is an independent organ of the protoplast. The nucleus consists of a relatively faintly staining ground mass in which the chromatin is embedded; the nucleus also contains a larger or smaller number of "central granules" which are never found outside the nucleus. The nucleus differs from that of higher plants in having no nuclear membrane or nucleolus and in its form. The cytoplasm contains numerous chromatophores, cyanophyceæ granules (Cyanophycinkörner), glycogen and vacuoles. The chromatophores contain chlorophyll, carotin and phycocyan. The product of assimilation is glycogen; starch is not demonstrable. The membranes of the vegetative cells consist principally of chitin, and those of the heterocysts consist largely of cellulose. The oft discussed central body is a genuine nucleus. During mitosis a spirem is formed which breaks up into a somewhat definite number of chromosomes. The phases resemble those of the higher plants, and the author refers to them as the spirem, equatorial plate dyaster and dispirem. Threads resembling a spindle are described and figured.

The writer believes that chromatin is a constant constituent of the cells of the Bacteria as well as of those of the Cyanophyceæ, and believes that the Cyanophyceæ and Bacteria are very intimately related.

The methods are described in full, but are too technical to be condensed

within the limits of a review. A table showing the behavior of the cell constituents when treated with various reagents and stains, is an important part of the work. These reactions as well as the morphological structures are clearly illustrated in the ten colored lithographic plates.

C. J. C.

**Waters, Campbell E.** Ferns, a manual for the Northeastern States with analytical keys based on the stalks and on the fructification. 8vo. pp. ix + 362. Illustrated. New York, Henry Holt & Co. Price, \$3.

This elegant volume is intended, primarily, for the amateur botanist and, consequently, is as free as possible from technicalities. The following

table of contents indicates, partially, the scope of the work: Introduction; reproduction of ferns; classification of our ferns; analytical key, based on the fructification; analytical key, based on the stalks; ferns of the polypody family; ferns of the curly grass family; ferns of the flowering fern family; ferns of the adder's-tongue family; fern photography; glossary; index. The analytical key based upon the stalks is an interesting piece of work and deserves to be tested by professional botanists in all parts of the country. The photographs of sori, most of which are taken at a magnification of  $5\frac{1}{2}$  diameters, are particularly excellent and will be valuable not only to the beginner who is learning to identify ferns, but also to the teacher who will find them excellent for demonstration. The book is intended to cover the same territory as Gray's "Manual," all the ferns being described and illustrated.

The detailed directions for photographing ferns can hardly be condensed to the limits of a review. It can only be mentioned that in photographing sori, at camera with a bellows extension of twenty-four inches was used, and that the focal length of the regular lens was reduced by slipping on over it a cheap "copying and enlarging" lens, thus giving a magnification of about  $5\frac{1}{2}$  diameters.

While the book is addressed to amateurs and is written in popular style, the author's extensive knowledge of ferns in the field together with the peculiar key and excellent illustrations—all of which are original—will make it useful to the experienced botanist.

C. J. C.

**Coulter, J. M., and Chamberlain, C. J.** Morphology of Angiosperms. (Morphology of Spermatophytes, Part II.) 8vo. pp. x + 348, 113 figures. New York, D. Appleton & Co., 1903. Price, \$2.50.

This work is similar in plan and scope to the volume on Gymnosperms by the same authors. The immense literature of the subject is organized and

presented, but the work is not a mere compilation, since it embodies the views and results gained by the authors in years of investigation in this line. It is hoped that the book will be useful to those who desire a more intimate knowledge of the morphology of the higher plants, and especially useful to those engaged in research. The titles of the chapters are as follows: Introductory, the flower, the microsporangium, the megasporangium, the female gametophyte, the male gametophyte, fertilization, the endosperm, the embryo, classification, geographic distribution of Angiosperms, fossil Angiosperms, phylogeny of Angiosperms, comparative anatomy of the Gymnosperms and Pteridophytes, comparative anatomy of Angiosperms. The last two chapters were written by Prof. Jeffrey of Harvard.

C. J. C.

v. Wettstein, Dr. Richard R. Handbuch der Systematischen Botanik. II Band; 1. Theil, with 100 text illustrations and one colored plate. 8vo. pp. 1-160. Leipzig und Wien. Franz Deuticke, 1903. 6 marks.

The second part of Wettstein's textbook has just appeared. (For review of Part I see JOURNAL OF APPLIED MICROSCOPY, 4: 1460-61, 1901. The

present installment deals with Bryophytes, Pteridophytes, and Gymnosperms; the remaining section, which is to deal with Angiosperms, will contain the index and will complete the work. The title is, *Hand-Book of Systematic Botany*, and the author proposes a complete system of classification, the main features of which were given in the review just referred to. The taxonomic characters of the larger groups, of families, and sometimes of the most important genera, are given and the classification determines the order of treatment. However, the book is of equal interest to the morphologist, for development and embryology are carefully treated and are constantly used to support the author's views of relationships. The illustrations representing the development of organs from the standpoint of comparative morphology are particularly instructive, as is also the plate illustrating the evolution of plants from the Algæ up to the Angiosperm. Considering the able manner in which the morphological part of the work is treated, one is hardly prepared for the statement that in Gymnosperms there is no alternation of generations, although traces of alternation are demonstrable, while in Angiosperms the reduction of the prothallium has proceeded so far that sure homologies can no longer be shown and the alternation of generations has entirely disappeared. Of particular interest are the introductory pages on the evolutionary composition of the Cormophytes, the homologies between the groups of Cormophytes and the causes of the changes in the homologous organs of the Cormophytes.

The Bryophytes are subdivided, as usual, into Musci and Hepaticæ; in the Pteridophytes three groups are recognized, the Filicinae, Equisetinæ, and Lycopodinæ; the Gymnosperms are subdivided into six classes, Cycadinæ, Bennettitinæ, Cordaitinæ, Ginkgoinæ, Coniferæ, and Gnetinæ.

The remaining part, dealing with Angiosperms, will be awaited with interest.

C. J. C.

Stevens, Frank L., and Stevens, Adeline C. Mitosis in the primary nucleus in *Synchytrium decipiens*. Bot. Gazette, 35: 405-415, pls. 16-17, 1903.

The cell of the host, *Falcata comosa* L., is about  $15\mu$  in diameter when the fungus parasite enters, but a rapid growth then begins and the host cell

soon reaches a diameter of  $100\mu$ , the entire space becoming filled by the enlarging parasite, which, however, remains in the unicellular condition. The nucleus of the parasite grows until it reaches a diameter of  $35\mu$ . After reaching this extreme size—the greatest recorded for the nucleus of a fungus—the nucleus gradually diminishes in size, and at the time of its first division is not more than  $10\mu$  in length.

Dangeard and Rosen, both of whom studied an allied species, *S. Tiraxaci*, claim that the first nuclear division is amitotic, and both agree that in the succeeding divisions both amitosis and mitosis occur. The present paper describes the first division in great detail and shows clearly that it is mitotic and that in the late anaphases it agrees well with mitoses in other fungi, particularly with *Albugo*. The behavior of the chromatin and the formation of the spindle are so peculiar that divergent accounts might be expected.

C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE MOODY.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole Moody, 125 Belvedere Street, San Francisco, Cal.

**Flint, J. M.** A new Method for the Demonstration of the Framework of Organs. *Johns Hopkins Hospital Bull.* **13**: 48-52, 1902.

Following Spalteholz's method with certain modifications the author obtained very satisfactory results from

the new process of digestion by the use of a digestive ferment. Pieces about 3 mm. thick of fairly large blocks of hardened tissue are best. These are cut so as to have 2 parallel planes. Van Gehuchten's fluid was perhaps the best as it works rapidly and gives good cytological pictures. Moreover, the chloroform in it loosens the fat. The mixture is: glacial acetic acid 10 parts, chloroform 30 parts, absolute alcohol 60 parts. Corrosive acetic gives good results with graded alcohols, but formalin, chromic acid or its salts and osmic acid are unsatisfactory. Trypsin cannot digest tissue hardened in fluid containing these reagents. After fixation dehydration should follow to avoid shrinkage, also gradual changes of alcohol. When dehydration is over the tissue is dropped into a Soxhlet apparatus and the fat extracted for 5 to 6 days continuously. To avoid possible explosions of the ether in this apparatus it is placed under a hood and heated by means of hot water from a Fletcher heater. After extraction for a week the tissue is removed and slowly passed through graded alcohols, which should be free from bubbles when poured on the tissues. The pieces are then washed in running water for 24 hours, and then put into the digestive mixture. Parke, Davis & Co.'s pancreatin can be used, but Grüber prepares a mixture which is more effective, rendering the sections more transparent. But a small quantity of pancreatin is used, enough to cover the end of an ordinary scalpel handle dissolved in 100 c. c. of a .5 per cent. solution of sodium bicarbonate. Since the products of digestion inhibit the action of the enzyme a dilute solution of trypsin should be used and frequently renewed. Changing the fluid every 48 hours is satisfactory for digestion. Enough chloroform must be used to prevent putrefaction, it should cover the bottom of the vessel in which digestion occurs. Thymol prevents decay but stains tissues a dirty brown. The heat required by digestion volatilizes the chloroform and fills the tissues with small bubbles causing it to float in the fluid. By making a small elevated stand of thick paper the tissue can be kept away from the bottom free from the chloroform and also the falling debris of digestion. The fats present in combination with protein can only be extracted after digestion, so that each process best be carried out at least twice, in some cases four times are required. Organs digesting quickly are thyroid, spleen, lymph gland, lung; while salivary glands, pancreas and adrenal are more resistant. The pancreas yields more slowly to its own ferment than any other. This of course applies only to hardened tissue; fresh was in no case used.



Pathological glands are equally well digested and changes in framework shown. When under the microscope no cellular debris can be seen, the tissue is washed thoroughly in water and cleared in glycerine. A piece of organ prepared in this way keeps its form perfectly and the capsule, finer connective tissue strands, vessels, ducts, and relation of lobules and follicles can be clearly traced. Even casement membranes like a fine web can be seen. Changes in light bring out many details. After this study is complete the glycerine can be washed out in water, the tissue embedded in paraffine and sections cut from  $4\ \mu$  upward. These may be stained with iron hematoxylin, fuchsin, nigrosin, Mallory's method or aniline blue alone. By using the immersion lens, the finest details in the arrangement of the fibers can be seen. Thicker sections in celloidin are even better than paraffine, cut from 15 to  $80\ \mu$  thick and stained with 8 per cent. acid fuchsin. Prolonged washing clears up the celloidin but leaves the fibres colored. The method is slow, the time required being 1 to 3 months for completed preparations, but yields very satisfactory results. Mall's frozen section method will still serve for rapid work.

A. C. M.

**Flint, J. M.** Note on the Framework of the Thyroid Gland. Johns Hopkins Hospital Bull. 14: 33-35, 1903.

This gland yields beautiful results by the method already described (above).

The specimen is a clear skeleton of the original block of tissue. Larger structures, as blood-vessels, even suggestions of follicles can be seen with the naked eye. No lobules or groups of follicles are seen; the blood vessels and their connective tissue make up the densest part of the framework. Proportionally there is more interfollicular connective tissue in the human gland than that of the dog. The follicles and the reticulated basement membranes appear heavier. The thyroid of the monkey has all the characteristics of the organ in the dog, the only difference being in the greater size of the follicles, being nearly twice those of the dog. In certain specimens the parathyroid is seen. This in dog and monkey is enclosed in the general capsule, but is separated by a distinct fibrous capsule. Staining the blocks of tissue  $150\ \mu$  thick with anilin blue brings out finer fibres with great clearness. No evidence of rupture is seen in any of the follicles, and the meshes are certainly large enough to allow free passage of fluids.

A. C. M.

**Marpmann, G.** New Medium for Mounting Microscopical Preparations. Zeitschr. angew. Mikr. 9: 1-3, 1903. (Rev. J. R. M. S. 4, 560, 1903.)

The author considers acetyl-cellulose an ideal medium. It is prepared by treating hydrocellulose with 3 per cent. sulphuric acid at  $70^{\circ}\text{C}$ . and after-

wards with acetic acid. On the addition of water acetyl-cellulose separates, and when dried forms a sandy powder, easily soluble in chloroform, nitrobenzol, etc. Excellent material is now on the market. A good cellulose solution keeps for a long time and may always be freshened by adding chloroform. Preparations are removed from alcohol, xylol, or one of the oils (cedar, clove, origanum) to a drop of the solution which has been placed on the slide. Another drop is added, and after arrangement with a glass rod, cover-glass is put on. The cover may be left off, which fact forms the most important advantage of the medium. Cover slips made from the following solution may be substituted for the glass: 10 parts acetyl-cellulose, 1 part aluminum palmitate, 15-20 parts chloroform, 1 part nitrobenzol. This solution is smeared on a plate of glass until it forms a layer about .15 mm. thick. When dry it can be peeled off in strips and cut up into suitable sizes.

A. C. M.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

Drzwecki, W. Ueber vegetative Vorgänge im Kern und Plasma der Gregarina des Regenwurmhodens. Arch. f. Protistenkunde 3: 107-126, Taf. 9, 10, 1903.

Material for study was secured from the testis of the earthworm (*Lumbricus agricola*) in March-July and fixed in picro-acetic acid mixture or in a mixture of one-half per cent. each of chromic and osmic acids combined with Perenyi's fluid in the proportions of 2:3. Living material was examined in the blood of the earthworm giving excellent opportunities for observing nuclear degeneration. Borax carmine and Delafield's hæmatoxylin were used as stains and most of the results were obtained from material thus stained, teased and mounted in clove oil or thin glycerine.

C. A. K.

Steuer, A. Ueber eine Euglenoide (*Eutreptia*) aus dem Canale grande von Triest. Arch. f. Protistenkunde 3: 126-138, mit 13 Textfiguren, 1903.

The organism formed an intense green water-bloom in the salt water of the grand canal on June 30-July 1, constituting a temporary monotonic plankton. Numbers decreased rapidly after July 2, and the species disappeared by July 13. Material was examined in the living condition, and also fixed in sublimate and in Flemming's solution, stained in Grenacher's or in iron hæmatoxylin and mounted in glycerine, clove oil or balsam.

C. A. K.

Plehn, M. *Trypanoplasma cyprini* nov. sp. Arch. f. Protistenkunde 3: 175-181, Taf. 12, 1903.

This parasite of the blood plasma was found in the German carp in a few individuals, often abundantly and at times only sparingly. Carp kept in aquaria and inoculated with the disease become anaemic when badly infested and the gills become pale. In extreme cases only a few drops of a watery scarcely reddish blood can be obtained from the host.

Streak preparations of the fresh blood which is infested will show a dozen or more parasites in a single field of the immersion lens, but owing to the rapid motion of the flagellates no observations can be made on their structure. As the preparation slowly dries up the motion slackens and the moribund individuals afford the only means of examining the living specimens. The parasite is very resistant, living for a day and continuing its movements in the body of the host, in normal salt solution or in a cover-glass preparation, if kept from drying up, and they have been known to live even for two days in a neutral red solution. The flagella do not stain in this dye, and the nucleus takes only a faint tinge at death. Vital staining with methylen blue was very successful, but the parasites are killed by it in about 30 minutes. The flagella stain deeply in this dye and are readily demonstrated by allowing the stain to act for a few minutes and then fixing the preparation in fumes of osmic acid for a few seconds.

These preparations do not reveal the nucleus, are best examined in water and may be subsequently stained. One method of staining proved successful. Dry preparations fixed for 15 minutes in absolute alcohol were stained in Romanowsky-Ziemann's methylen blue-eosin. The nucleus takes a deep blue-violet color and the so-called centrosome appears as a mass of reddish granules. The drying process destroys the small flagellum and distorts the form of the body.

C. A. K.

**Bezenberger, E.** Ueber Infusorien aus asiatischen Anuren. Arch. f. Protistenkunde 3: 138-175, Taf. 11 und 23 Textfiguren, 1903.

Material for this study was secured from living *Anura* and was examined in living condition in normal salt solu-

tion, fixed in Schandinn's sublimate-alcohol mixture which likewise fastens the animals to the substratum, slide or cover-glass. Picro-carmin was used as a stain for *in toto* preparations. Portions of the intestine and its contents were sectioned in  $5\ \mu$  sections and stained in paracarmine or hæmatoxylin-eosin. Sections  $1$  to  $2\ \mu$  in thickness stained in iron-hæmatoxylin were used for fine details of structure. The foreign matter and intestinal mucus was removed from *Opalina ranarum* by shaking the intestinal contents vigorously in a tube with normal salt solution and then removing the isolated parasites to fresh fluid in which they were again shaken. They were finally rid of the foreign matter by allowing them to filtrate through a loose-meshed wad placed a few centimeters above the bottom of a cylinder of water. *Intra vitam* staining was accomplished successfully by the use of moderately strong solution of Romanowsky's soda methylen blue prepared after the method of Ruge for the malarial parasites. Not all of these parasitic infusoria could be stained in this fashion, some died quickly in the stain and other species resisted its action.

C. A. K.

**Stevens, N. M.** Further Studies on the Ciliate Infusoria, Lichnophora and Boveria. Arch. f. Protistenkunde 3: 1-44, Taf. 1-6, 1903.

Conjugating individuals of *Lichnophora* found upon their hosts *Thysanozoon*, *Asterina* and *Ophiothrix*, when placed

upon a glass slide attach themselves to the glass and remain in the one place until the gametes separate. The process of conjugation continues for more than 17 hours. It was found to be possible to fasten these conjugating pairs to the slide for subsequent treatment in staining, etc., by fixing them in absolute alcohol + 5 per cent. glacial acetic acid. Fixing fluids containing osmic acid caused *Lichnophora* to loosen its hold upon the glass and corrosive-acetic permitted them to fall off in the alcohol. Maupas's glycerine methods were not successful with this form, and the micro nucleus was difficult to stain in anything but iron-hæmatoxylin in sections. Whole material was stained for 12 hours in alum carmine and decolorized in acid alcohol.

C. A. K.

**Francis, E.** An Experimental Investigation of *Trypanosoma lewisi*. Bull. No. 11, Hyg. Lab. U. S. Pub. Health & Mar. Hosp. Serv. Wash., pp. 1-26, 4 pls. 1903.

*Trypanosoma lewisi* occurs in the blood of the wild gray rat of Europe and the sewer rat, but was not found in rats captured in Washington. White and

spotted rats were inoculated and the parasite was studied in fresh and stained preparations of the blood. Fresh blood in the hanging drop at room temperature and at  $37^{\circ}\text{C}$ . though carefully examined failed to yield any other stages of

the parasite than those present when the blood was first drawn. Examinations of blood drawn at short intervals from a rat after intraperitoneal inoculation will provide the entire cycle of development. In white rats the infection lasts from seven to fourteen days. The ordinary anilin and hæmatoxylin stains fail completely in staining *Trypanosoma*, but the Romanowsky, the Goldhorn, and the Jenner stains all gave most excellent results. Wright's modification of the Romanowsky method was employed as follows: One per cent. methylene blue is added to  $\frac{1}{2}$  per cent. solution of sodium bicarbonate and the mixture is steamed in an Arnold sterilizer for one hour. When cold, eosin is added till the color changes from blue to purple and a metallic scum is formed on the surface. The black precipitate which forms is collected on a filter, dried, and dissolved in methyl alcohol. The blood films spread evenly, are dried in the air and are then covered with the alcoholic solution of the dye for one minute. Water is then added drop by drop to the preparation until the dye becomes semitranslucent and a yellowish metallic scum forms on the surface. This mixture remains on the preparation for 2 to 3 minutes. The preparation is then washed in distilled water until the film has a yellowish or pinkish tinge, is dried between filter papers and mounted in balsam.

C. A. K.

**Prowazek, S.** Flagellatenstudien, Anhang: Fibrilläre Strukturen der Vorticellinen. Arch. f. Protistenkunde **2**: 195-212, Taf. 5, 6, 1903.

*Mastigameba, Cercomonas, Monas, Bicosæca, Chilomonas, Polytoma, and Vorticella* were fixed in quantity in Flemming's fluid, condensed by the use of

a Cori's hand centrifuge, and by its help carried through the usual technique preliminary to embedding in paraffin. The concentrated material was brought from xylol into a trough made in a watch glass full of cold paraffin and then placed in a warming oven and embedded. Sections 2 to 3  $\mu$  in thickness were stained in iron hæmatoxylin.

C. A. K.

**Taylor, J. R.** Observations on the Mosquitos of Havana, Cuba. Reprint from La Revista Med. Trop., June, 1903, 27 pp.

This paper contains a number of suggestions of value to those who wish to rear mosquitos for the purpose of study.

Eggs deposited at night usually hatch out early in the day so that search for them should be made in early morning hours. A white enamelled ladle or dipper is very useful in examining the margins of pools and other bodies of water, for eggs, larvæ, or pupæ. Larvæ may be raised in the laboratory in small aquaria supplied with debris from the bottom of the breeding pools. A small amount of raw rice or bread may also be added to increase to amount of food in the aquaria, but care must be taken to prevent fouling or stagnation, by changing the water when necessary. When the pupæ appear they should be changed to small dishes and then placed in the gauze covered breeding cages. Large and airy breeding cages should be provided if eggs are desired from captive females. Copulation occurs soon after emergence from the pupal stage, and most captured females have been fertilized and will lay fertile eggs if provided with a meal of blood. For this purpose the hand may be introduced into the breeding cage, or a pigeon or rabbit may be placed in the cage during the sleeping hours of the animal. The eggs are laid within a day or two after the meal.



Battery jars with gauze cover held in place by strings of adhesive plaster were ordinarily used for keeping mosquitos in confinement. A small dish of water containing some living grass is placed in the jar and food is supplied in the form of lump sugar hung in a gauze bag at the top of the jar. In the hot moist climate of Havana this was found to be preferable to banana, often recommended as a food for mosquitos. Watermelon, apples or other fruits may also be used. In such jars *Culix* has been kept alive for more than five months. C. A. K.

**Hamburger, C.** Beiträge zur Kenntnis von *Trachelius ovum*. Arch. f. Protistenkunde 2: 1-32, Taf. 1, 2, mit. 4 Textfiguren, 1904.

*Trachelius* is one of the largest and also one of the rarest of the infusoria.

It was found near Heidelberg in shaded ponds full of fallen leaves in September-November. Leaves and pond water were brought into the laboratory with a gradual transition from field to room temperatures. In a few days the sides of the culture dishes were covered with a heavy growth of *Epistylis*, among which *Trachelius* was soon to be found in abundance feeding upon its zooids.

Preservation of *Trachelius* is difficult on account of the large amount of water in the animal. Sublimate, sublimate-acetic and formol all failed, but osmic vapor in 1 per cent. osmic acid preserved not only the bodily form but also the internal structure. To prevent blackening the osmic acid was washed out by water after a few minutes. Flemming's and Hermann's fluids also gave excellent results. Ectoplasmic structures were demonstrated in osmic material by adding a few drops of 5 per cent. aqueous solution of sodium bicarbonate and allowing the solution to concentrate by evaporation. When the demonstration reaches the desired point the solution is replaced by dilute glycerine. The clearing and embedding processes were carried on in small glass cylinders 2 x .5 cm. whose lower opening was closed by fine bolting cloth. This container can be readily passed through vessels of chloroform, chloroform-paraffin and melted paraffin and then embedded. When the paraffin cast is cold the cylinder can be cut out, the silk diaphragm removed and the paraffin cylinder pushed out from the slightly warmed glass tube. This method is readily applicable to other small objects. C. A. K.

**List, T.** Die Mytiliden des Golfes von Neapel und der angrenzenden Meeres-abschnitte. Fauna u. Flora d. Golfes v. Neap. Monogr. 27: 312 pp, 17 figs. u. 22 Taf. 1902.

The animals were narcotized by the addition of 2 per cent. cocain in sea water, the amount being gradually increased. Care must be taken, espe-

cially with young individuals, not to prolong the narcotization until maceration begins. When the whole animal is to be fixed a piece of wood or cork is placed between the valves of the shell before the fixing agent is added. If individual organs only are to be fixed these are dissected out under water. If the whole, or a part of the shell remains attached the fixing agent should contain some free acid. For anatomical preparations 70 per cent. alcohol + 2 to 3 per cent. nitric acid, or chrom-acetic mixture were used. For histological preparations strong Flemming's fluid or Mayer's picro-nitric mixture was used. Sublimate, with or without acetic acid, gave good results. Cilia were well preserved by 10 per cent. formol in sea water for 10 minutes followed by one of the above named fixing

agents. Sections were stained in Mayer's hæmalum, which gives a fine nuclear differentiation and stains also the mucous glands. Eosin in aqueous and in alcoholic solutions, with and without a trace of acetic acid, was used as a plasma stain. The acetic-eosin was found to demonstrate in some instances the nerve fibers and finest nerve fibrillæ when employed as follows: The sections after staining in hæmalum were flooded with distilled water, to which 2 drops of acetic acid had been added, and after a few minutes exposure, the water is poured off and a few drops of an aqueous solution of eosin was added. A precipitate is formed which is washed off after a few moments, and the slide is then soaked in distilled water, dehydrated quickly in absolute alcohol and mounted in balsam. The peripheral nervous system was demonstrated in total preparations from underfed animals in whose nerves a granula is formed which blackens readily in osmic acid. The narcotized mussels are fixed in Flemming's stronger mixture for several days, the shell removed, the soft parts washed in water, and passed through alcohol grades in bright sunlight. In the course of a few days this brings about an intense blackening of the nerves, which can then be followed readily in material cleared in zylol or benzole. The course of the more centrally placed nerves may be followed by dissection of the cleared material. The course of the digestion tract was demonstrated upon animals in which the sexual glands were not developed by feeding them upon finely ground india ink, which quickly fills the intestine and begins to invade the liver. The animals are narcotized before this invasion proceeds very far, are fixed in nitric acid-alcohol or picro-nitric mixture. When cleared in zylol the course of the digestive tract is plainly demonstrated.

C. A. K.

**Tower, W. L.** The Origin and Development of the Wings of the Coleoptera. *Zool. Jahrb. Abth. f. Anat. u. Ont.* 17: 517-572, Taf. 14-20, and 8 figs. in text, 1903.

None of the methods usually recommended for insect tissues gave reliable results in all cases. Perenyi's fluid used hot or cold, sublimate, picric or

chromic acid mixtures were unsatisfactory. Hermann's and Flemming's fluids gave excellent results with small pieces or young larvæ, but were not adapted to old larvæ or large pupæ. The following sublimate-acetic mixtures were devised and are stated to give results exactly like those given by Hermann's and Flemming's fluids:

	No. 1	No. 2	No. 3
Saturated solution of corrosive sublimate in 35 per cent. alcohol	70	95	60
Glacial acetic acid	25	2	10
Nitric acid c. p.	5	3	—
Platinic chloride 2 per cent. in distilled water	—	—	30

Number 1 is used for large larvæ or pupæ, heated to 80° C. in a closed flask and poured suddenly over the specimens and allowed to act for 2 to 5 minutes. It is then replaced with No. 2 and the temperature is maintained at 30 to 40° C. for several hours. After removal from No. 1 the insect should be cut in several pieces with a knife. Small larvæ and pupæ should be fixed in No. 2. Number 3 was the most generally useful reagent, giving excellent results when used either warm or cold, but it should be applied only to small pieces of tissue. After

killing dehydrate rapidly, clear in cedar oil and preserve in paraffin. Insect tissue loses its finer structure if preserved in alcohol and becomes more difficult to section. No method of softening chitin was found which left the tissues uninjured. Proper fixation, careful embedding, sharp knives, and patience are necessary for good sections of hard chitinous structures.

C. A. K.

**Eycleshymer, A. C.** The Early Development of *Lepidosteus osseus*. Dec. Pub. Univ. Chicago, 10: 259-276, pls. 17, 18, 1903.

The fish were taken by spearing in lakes in Michigan and Wisconsin in June, and the eggs were artificially fer-

tilized. Eggs and sperm obtained by stripping were more favorable than those obtained by excision. Formalin in 8 to 10 per cent. aqueous solution followed by hæmalin surface staining clearly defined the cleavage grooves; chrom-acetic to which a little 1 per cent. osmic acid had been added also gave most excellent results. Material killed in corrosive-acetic was most satisfactory for sectioning, and for section staining. Picro-acetic and picro-sulphuric while fairly good for larval stages caused distortion and swelling of early stages obscuring the cleavage furrows.

C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory, University of Michigan, Ann Arbor, Mich.

**Loeb, J.** Ueber die Befruchtung von Seeigeliern durch Seesternsamen. Arch. f. d. ges. Physiol. 99: 323-356, 1903.

As a result of his experiments on the cross-fertilization of sea-urchin eggs with star-fish sperm, Loeb finds that

the composition of the solution in which the eggs are placed is the determining factor for successful crossing. In ordinary sea water or in a van't Hoff's solution (a solution with the following substances in the proportions indicated: 100 NaCl, 7.8 MgCl<sub>2</sub>, 3.8 MgSO<sub>4</sub>, 2.2 KCl, 1 CaCl<sub>2</sub>) to which has been added 0.1 to 0.2 ccm.  $n/10$  NaOH, or 0.4 to 2 ccm.  $5/8$   $m$  NaHCO<sub>3</sub>, per 100 ccm. of the solution, eggs of *Strongylocentrotus purpuratus* can be easily fertilized with the sperm of the same species. In these solutions it is not possible to fertilize any, or at best but very few of these eggs with star-fish sperm (*Asterias ochracea* was the form used). If, however, there be added to the van't Hoff solution a slightly larger amount of sodium hydroxide (0.3 to 0.4 ccm.  $n/10$  NaOH per 100 ccm. of the solution) the eggs are fertilized by the star-fish sperm quickly and in large numbers. The experiments show that besides the NaOH (or hydroxylions) Ca and Na ions are necessary both for the ordinary fertilization and hybridization. The only anions necessary beside the OH ions are Cl ions. Aside from the principal results here stated some of the details of this work, as brought out by the author, are very interesting and suggestive, as for example the fact that the hybrid larvæ formed skeletons, this being of course a purely sea-urchin character. Lack of space forbids further mention of this interesting and important work here.

R. P.

Klug, Ferd. Zwei Froschherz-Manometer als Kreislaufschema und Versuche mit denselben. Arch. f. d. ges. Physiol., 99: 594-618, 1903.

Prof. Klug has devised and describes under the above title some very ingenious devices for demonstrating and studying the action of the heart under varying conditions. The simplest, and perhaps most generally useful of these pieces of apparatus is shown diagrammatically in Fig. 1. The different parts are mounted on a glass plate as a base. Two metal cannulæ of different sizes are passed through a rubber stopper (*a*).

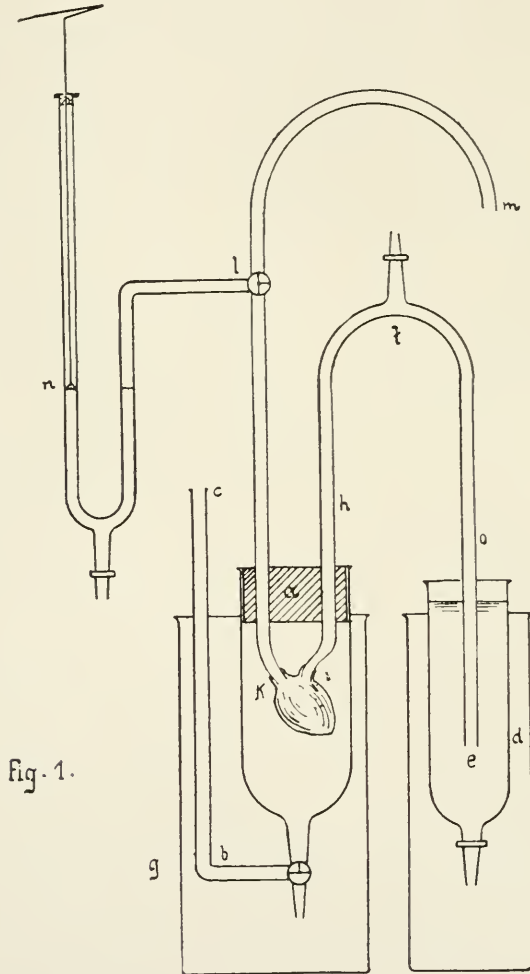


Fig. 1.

The larger of these cannulæ is inserted and tied into the sinus venosus of a surviving frog heart. The smaller cannulæ is inserted in the left aorta. All the other vessels of the heart are ligatured and the heart is removed from the body. The heart so prepared is then suspended in a glass vessel containing .6 per cent. NaCl solution, the vessel being closed above by the rubber stopper. From the lower portion of this vessel passes a volumetric tube (*b*) by means of which the



changes in the volume of the heart may be measured. This whole portion of the apparatus lies in a larger glass vessel (*g*), through which water of a constant temperature may be circulated, when it is desired to study the effects of different temperatures on the heart's action. The vessel (*e*) serves as a blood reservoir from which the heart takes oxygenated blood through the venous tube *efh*. The blood reservoir is again placed in a larger vessel (*d*) in order that the temperature may be controlled. Through the cannula *i* the blood is brought into the sinus venosus, while the cannula *k* conducts it from the left aorta out into the arterial glass tube *km*. This arterial tube has at *l* a T-cock and a branch tube leading off to the manometer tube *n*. By means of this manometer the blood pressure at each stroke of the heart may be measured. The arterial tube proper ends at *m*, and from this opening the blood drops freely back into the blood reservoir (*e*). This opening *m* stands 11cm. above the heart, while the reservoir (*e*) can be raised or lowered as desired. The venous tube from *o* to *e* is made of rubber tubing so that the amount of blood passing to the heart may be controlled. The reservoir may, of course, be filled with any nutrient fluid whose action on the heart it is desired to test. The author finds that the best results are obtained with defibrinated arterial blood. The apparatus serves admirably for the demonstration of the phenomena of the circulation, as well as for investigations on the heart.

R. P.

Holmes, S. J. Sex Recognition among Amphipods. Biol. Bull. 5: 288-292, 1903.

Certain of the amphipod Crustacea have the following curious habit connected with reproduction; for some time before the eggs are laid the male tightly clasps the female and carries her about with him. The purpose of the habit is probably merely to ensure the close proximity of the sexes at the time of egg laying in order that the eggs may be properly fertilized. The problem to which Holmes has addressed himself is this; how do the males recognize the females? The account given is based on the reactions of *Hyallella*. It is found that during the period of clasping the female is remarkably impassive, keeping the body as compactly drawn together as possible, and taking little or no part in the swimming. The female as soon as seized by the male curls up and allows herself to be rolled about without a show of resistance or protest. By blackening the eyes of some males it was shown that sight plays no part in sex recognition, as these blinded males were soon provided with mates. That the sense of smell is not an important factor in this recognition was shown in two ways. In one experiment the first antennæ, in which the sense of smell is localized, were removed from several males, and these mutilated individuals were then put into a dish with females. In all cases the males seized the females and carried them about just as normal males would have done. In another experiment the males and females in the same dish were separated by a wire gauze partition. Under these conditions the males paid no attention to the females, although as soon as the partition was removed the males quickly acquired partners. It was found by close observation of normal animals that the males do not pursue the females, and that the determining factor in sex recognition is found in the reactions of the individuals when they accidentally collide in swimming about. If a male

collides with a female the latter becomes quiet at once and the male grasps her. If two females collide both immediately become quiet, but as as neither is clasped they soon start swimming again. Finally if two males collide each tries to seize and clasp the other, and, of course, on account of their struggles neither is successful. It was shown experimentally by cutting off the large second gnathopods (the principal means of defense) from males and then putting these mutilated specimens into a dish with normal males, that such mutilated and defenceless males were carried about by the others exactly as if they had been females. So it appears that practically the sole method by which sex recognition is secured among the Gammaridea depends on the reactions of individuals when they come into contact with other individuals of the same species.

R. P.

**Axenfeld, D.** Invertin in Honig und im Insek-  
tendarm. Zentralbl. f. Physiol. 17: 268 and  
269, 1903.

Starting from the fact that in the residue remaining after dialyzing honey invertin is present, Axenfeld has found a ferment capable of inverting cane sugar in the intestine of a number of species of insects. Such a ferment is present in the intestine of the wasp, *Musca carnaria*, many *Lepidoptera*, including *Pieris*, *Vanessa*, and the caterpillar of *Carporapsa pom.* Among the *Coleoptera* it was found in *Carabus*, *Dyticus*, *Melolontha*, *Notonectes* and *Hydrophilus*. It was also found in *Cicada com.*

R. P.

**Folin, O.** On Rigor Mortis. Amer. Jour.  
Physiol. 9: 374-379, 1903.

The object of this work is to prove that the prevailing view as to the cause of a muscle going into a state of rigor mortis is incorrect. It is generally held that rigor mortis is due to the coagulation of some substance in the muscle plasma. Folin shows that this view is untenable, by producing rigor mortis in frog and fish muscle by lowering the temperature to  $-15^{\circ}\text{C}$ . There is absolutely no evidence that any coagulation takes place under the circumstances. Yet the rigor mortis produced is typical.

R. P.

**Kobert, R.** Ueber einige Enzyme wirbelloser  
Thiere. Arch. f. d. ges. Physiol. 99: 116-  
186, 1903.

In this paper the author sums up the results of his own and his students' work on enzymes present in invertebrate organisms. He was able to obtain enzymes in different organs of a variety of invertebrates, including representatives of practically all of the larger groups beginning with the *cœlenterates*. Nearly all the common sorts of enzymes were found. The paper forms an excellent reference work on the subject.

R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt,  
Harvard University Medical School, Boston, Mass.

**Blumer and Gordinier.** A case of chronic lymphatic leukæmia without enlargement of the lymph nodes. *Medical News*, 1903, LXXXIII, p. 833.

The clinical picture was that of pernicious anæmia. As in the similar case of lymphatic leukæmia without enlargement of the lymph nodes reported by Pappenheim and Reed, the striking feature of the pathological examination was the marked lymphoid change in the marrow of the long bones.

The predominating cell of the blood although resembling the normal lymphocyte, presented points of difference of which the most important was the acidophilic character of its protoplasm. The writers regard this prevailing type of cell as identical with the predominating cell in Reed's case. They are inclined to accept the theory of Rubinstein that both lymphocytes and erythrocytes originate from a colorless mother-cell in the bone marrow, and they regard the predominating white cell in the blood of their case as this mother-cell. Arrest of development of this mother or ancestral cell would explain both the anæmia and the lack of typical lymphocytes noted in their case.

The writers held that lymphocytes in adults are formed normally both in the lymph nodes and in the bone marrow. Theoretically, therefore, it would be possible to have lymphatic leukæmia as a result of disease of the bone marrow, or of the lymph nodes, or of both. When lymphatic leukæmia is of the ordinary type, that is with enlargement of the lymph nodes, an increase of the typical lymphocyte occurs, whereas in the cases without enlargement of the lymph nodes the predominating cell differs from the ordinary lymphocyte and is formed in the bone marrow.

J. H. P.

**Weber, F. P.** Ein Fall von akuter Leukæmie, mit einem Schoma für die Einteilung der Leukæmien und Pseudoleukæmien. *Virchow's Archiv*. 1903, CLXXIV, p. 324.

Weber regards leukæmia as the result of a neoplastic activity of the leucocyte producing organs in which the tumor cells gain entrance into the blood stream. Hence the disease according to this theory should be grouped with the new growths. If the less differentiated cells of the type of the large lymphatics are drawn from the lymph nodes or from the bone marrow lymphatic leukæmia is the result. In the acute cases the blood usually contains more of the large than the small lymphocytes. If the more differentiated cells, that is the granular cells of the bone marrow, are concerned chiefly in the tumor formation, the pathological picture of myelogenous leukæmia is produced. According to this theory lymphatic leukæmia is a tumor arising from the proliferation of a more embryonic cell-type than myelogenous leukæmia, and this agrees with the fact that in its development and course lymphatic

leukæmia is generally more rapid. Leucocytosis or lymphocytosis is simply the expression of a tissue reaction while leukæmia always arises from the emigration of tumor cells from a hyperplastic neoplasm in the leucocyte tissues. If the organism attempts the rapid formation of myelocytes there may not be sufficient time for the development of granules in the cell protoplasm, and as a result unripe cells are thrown into the circulation which resemble the large and small lymphocytes and not the typical myelocytes. The writer holds that cells of the myelocytic type found in the spleen and lymph nodes in cases of myelogenous leukæmia may be formed in situ out of the undifferentiated large lymphocyte.

Weber reports a case of acute leukæmia of two months duration in which the blood picture was that of a mixed cell leukæmia (Reed's terminology). In a spread of the heart's blood 93 per cent. of the white cells were lymphocytes and 6 per cent. myelocytes. The liver, spleen, and lymph nodes were enlarged. Great numbers, both of lymphoid cells and eosinophilic myelocytes were found in the internal organs. Professor Muir of Glasgow, who examined the sections, regarded the case as one of lymphatic leukæmia, although he had never seen such numbers of eosinophiles in that disease.

J. H. P.

**Meyer.** Beiträge zur Leukocytenfrage. Münch. Med. Woch. 50: 1489, 1903.

Brandenburg in 1900 called attention to the fact that the blood in myelogenous leukæmia without the addition of  $H_2O_2$  or turpentine oil would color guaiac tincture blue. This held true even when very small amounts of blood were used. Bone marrow from a case of myelogenous leukæmia gave the reaction, but the structures rich in lymphocytes as the spleen, thymus, and lymph nodes as well as the other organs of the same individual yielded negative results.

Meyer found the reaction did not occur when the blood and bone marrow from a case of lymphatic leukæmia were tested. He confirmed Brandenburg's results in two cases of myelogenous leukæmia and modified the technique. The following simple method of applying the test is recommended: Two or three drops of leukæmic blood are collected in a test-tube and mixed with a large amount of water, so much in fact that no color of blood remains. The fluid becomes a deep blood on the addition of guaiac tincture.

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Under Professor Friedrich Müller's direction a study of this property of myelocytic blood was made. The reaction was found to depend upon an oxidizing ferment present in the myelocytes. The same ferment was demonstrable in pus cells and in the completely normal neutrophilic leucocytes, but not in the lymphocytes.

The author believes that the conception of an acute lymphatic leukæmia as a distinct variety of the disease is correct, and that it should be sharply differentiated from the myelogenous form.

Rosin and Bibergeil have shown that the nucleus of dead leucocytes is colored by neutral red while the nucleus of living leucocytes is not. The cytoplasm takes the vital stain.

Meyer found in a case of leukæmia that a relatively large number of nuclei were stained at the time when the excretion of uric acid was greatest. Hence



he concludes that the destruction of leucocytes can be estimated not only by the uric acid output but in the blood preparation by the number of leucocytes taking the nuclear stain with neutral red.

J. H. P.

**Billings and Capps.** Acute myelogenous leukæmia. *Amer. Jour. of the Med Sciences*, 1903, CXXVI, p. 375,

Until recently all cases of acute leukæmia were regarded as belonging to the lymphatic type of the disease. Fränkel,

who made a careful study of the literature up to 1895, did not admit the existence of an acute myelogenous leukæmia. Pinkus in his article in Nothnagel's System, published in 1901, was not able to record a single case. Billings and Capps give the details of an undoubted case of an acute myelogenous leukæmia observed by them in the analysis of seven cases collected from the literature. The duration of the disease in their case was two months. The number of leucocytes varied from 540,000 to 374,000 per c. mm. When first examined the blood showed 30 per cent. of myelocytes and 39 per cent. of large mononuclears. Later the percentage of myelocytes increased to 54.

The onset in most of the recorded cases was sudden. Four cases had an inflammation of the throat. The lymph nodes were usually somewhat swollen and the spleen was always palpable, although in only two instances greatly enlarged. The anæmia in every case was severe and progressive. The number of leucocytes ranged from 16,000 to 540,000. The average count was much lower than usually found in the chronic myelogenous leukæmia. There was difficulty in separating the mononuclears and the myelocytes due to the fact that many transitional cells with faint indistinct granules occurred. The myeloid cell has usually an eccentrically placed nucleus which is often oval and the protoplasm is frequently faintly granular.

When the large mononuclears are associated with a considerable number of myelocytes of the same size and when any of the mononuclears show indistinct granules the writers believe it is safe to regard them as myeloid cells.

In the recorded cases of acute myelogenous leukæmia the large mononuclears never formed less than 15 per cent. of the leucocytes in the blood, while the myelocytes varied from 6 to 60 per cent. Eosinophiles, mast-cells, and nucleated red corpuscles were present in varying number in some cases and absent in others.

J. H. P.

**Simon.** A Case of Myelogenous Leukæmia with several unusual Features (Absence of Eosinophilic Leucocytes). *Amer. Jour. Med. Sci.*, 125: 1903.

Ehrlich has asserted that in every case of myelogenous leukæmia there is an increase of the absolute number of

eosinophiles and this increase of eosinophiles is of great diagnostic value. This doctrine of Ehrlich's has been repeatedly but unsuccessfully attacked. Simon, however, reports a case in which throughout the course of the disease the eosinophiles were absent from the blood. He could find no similar observation in the literature, and he met with but one instance of leukæmia in which the total number of eosinophiles was not increased. The blood picture in Simon's case was remarkable in other respects. The percentage of mast cells was small, and early in the disease the absolute number was low. This is interesting, in view of Ehrlich's doctrine that the mast cells show an absolute increase in all cases of

myelogenous leukæmia and that this increase of mast cells is of even greater diagnostic importance than the increase of eosinophiles. Shortly before death, however, the total number of mast cells became markedly increased. Until near the end of the disease there was only slight enlargement of the spleen, and the total number of leucocytes scarcely exceeded the physiological limit. Myelocytes were present in the blood and the number increased with the advance of the disease. During the last six months of illness myelocytes formed from 30 to 50 per cent. of the leucocytes. This case and the one reported by L. Michaelis illustrate the fact that the appearance in the blood of myelocytes rather than an increase in the total number of leucocytes is the characteristic feature of the disease. Shortly before death the leucocyte count rose to 116,000.

Large mononuclear cells devoid of granules and distinctly different from the large mononuclear leucocytes of the blood appeared in large number toward the end of the illness when no less than 54 per cent. of all the leucocytes belonged to this type. Nägeli claims that these cells are found in the blood in every case of myelogenous leukæmia and he noted also that they may in the later stages of the disease form the predominating leucocyte in the blood. Nägeli regards them as specific bone marrow cells and the antecedents of Ehrlich's myelocytes. For the study of the mononuclear non-granular elements in the blood the eosinate of methylene blue should be used, as Ehrlich's triple stain does not differentiate the lymphoid cells.

J. H. P.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn, Wesleyan University, Middletown, Conn.

Among the many papers presented at the fourth meeting of the American Society of Bacteriology were the following on bacteriological methods, outlines of which may be found in *Science*, March 6, 1903, and in *Cent. f. Bact.* II, p. 381.

**Dunham.** The Influence of Physical Conditions on the Character of Colonies on Gelatin Plates.

Observations have convinced the author that the character of colonies in gelatin varies with conditions. The same bacteria may produce colonies wholly unlike and different species of bacteria may produce colonies indistinguishable from each other. This led him to testing the effect of varying the physical condition of the gelatin upon the colonies of *B. typhosus* and *B. dysenteriae*. He finds that the stiffness of the gelatin has a profound effect upon the character of the colonies, especially upon the deep lying colonies. In thin gelatin the colonies of typhoid have a tendency to form closely aggregated masses of small colonies which do not appear in denser gelatin and that other great differences appear. He finds, as would be expected, that the stiffness of gelatin varies with the grade of gelatin used, but also that it varies greatly according to the amount of heating which is received. Even the question of the cooling of the gelatin

plates by low temperatures or allowing it to cool at the ordinary room temperature affects the type of colony produced. In short, Dunham insists that in the study of bacterial colonies it is necessary to pay the most strict attention to the physical nature of the gelatin and use methods of insuring greater uniformity.

**Hastings.** Milk-agar as a Medium for Demonstrating the production of Proteolytic Enzymes.

The author suggests the use of milk agar as an advantageous medium for detecting proteolytic enzymes. He uses skimmed milk, which is added to common agar, and sterilized in the ordinary way. After cooling, this makes an opaque medium which may be used for slant tubes or petri dishes and upon which bacteria will grow luxuriantly. When cultures of liquefying organisms grow upon this medium it is rendered transparent by the digestion of the casein, and bacteria which produce enzymes may thus be very readily detected. The advantage of the use of this medium over the use of gelatin is chiefly that the bacteria may be cultivated at high temperatures and the enzyme forming character therefore determined with great rapidity. The material is also adapted for detecting the presence of enzymes in animal tissues, for these can be mixed with the milk agar together with a small amount of antiseptic for preventing bacteria growth. If enzymes are present the medium will be rendered transparent by the action of the enzymes.

**Copeland.** Summary of the Steps which must be followed in Staining Flagella by Loeffler's Method.

The author gives some useful details for rendering the method of flagella staining more uniform. He leaves the bacteria to be tested in suspension in water for one or two hours to dissolve away the gelatinous capsule. He insists on the use of the best tannic acid, iron sulphate, and basic fuchsin for a mordant. These are heated to between 70 and 75°, until the steam arises to a height of two inches. Then the preparation fixed on a cover-glass is placed in the mordant for half a minute. The stain which follows must be of the best aniline oil, pure alcohol, and saturated alcohol solution of basic fuchsin. It must be used cold and the preparation must be stained in it for 8 or 10 seconds. When thus used Copeland is convinced that Loeffler's method of flagella staining is superior to any others in use.

**Winslow.** Studies on Quantitative Variations in Gas Production in the Fermentation Tube.

Experiments with *B. coli* have shown the author the necessity of caution in the interpretation of the gas production in fermentation tubes. Using tubes of the same broth and inoculating with the same culture of *B. coli* Winslow found notable variations in the gas production. The amount of gas produced in 16 hours varies from 20 to 62 per cent. and the amount produced in 64 hours varies from 38 to 86 per cent. This is not wholly due to the rapidity of gas formation, for the maximum production in different tubes varies from 42 to 86 per cent. During the first 12 hours the amount of gas produced depends upon the quantity of inoculated material, and the amount of hydrogen is relatively greater than at a later period. Between 24 and 48 hours the gas corresponds to the classical formula 2:4; after this, there is a falling off in the total amount of gas, attribut-

able to the absorption of carbon dioxide. The author emphasizes, therefore, the necessity of more careful determination of the gas in interpreting the meaning of this test.

**Fremlin.** On the cultivation of the Nitroso-Bacterium. *Journal of Hygiene* III, 364, 1893.

Since the work of Winogradsky upon the nitro-bacteria it has been taken for granted that these organisms can grow only in the absence of organic foods and cannot be cultivated therefore upon any ordinary laboratory media. For their study it has been common to use a medium made of silica, the preparation of which is extremely difficult and unsatisfactory. Fremlin finds that it is possible to cultivate this organism upon gelatin and agar. He isolates them by inoculating soil into a solution of ammonium sulphate, one part; potassium phosphate, one part; magnesium carbonate, 10 parts; water, 1000 parts. In this solution the ammonium is slowly oxidized by the bacteria. After growth sub-inoculations were made and carried on for five successive sub-cultures. From these final cultures plates were made for the isolation of pure cultures. The use of silica proving difficult, he resorted to gelatin and agar, using not only the ordinary media, but also media made from sterilized soil extracts and stiffened with gelatin and agar. In all these media he found the organisms would grow and from them he isolated pure cultures without difficulty. He concludes, therefore, that these organisms will grow in the presence of organic material. He finds that in the presence of large percentages of organic material their power of converting ammonia into nitrate is decreased.

**Buchner and Meisenheimer.** Enzyme bei Spaltpilzgährung. *Ber. Deutsch. Chem. Ges.* XXXVI, p. 634, 1903.

Since the discovery of Zymase by Buchner it has been regarded that other fermentations might also be produced by the direct action of enzymes secreted by bacteria. The lactic and acetic fermentations have been studied by the authors. Their method of isolating the enzyme is as follows: They cultivate the organism (the lactic bacteria) in wort and after some days growth centrifugalize. The sediment is mixed with water and again centrifugalized, after which it is mixed with 20 parts of *acetone*. It is filtered and washed on the filter with acetone and ether and then dried in a vacuum. One liter of culture yields one gram of dry substance. This is mixed with an equal weight of quartz sand and rubbed with a little water. This forces out of the material a product which has the property of forming lactic acid from sugars even under aseptical conditions.

H. W. C.



## GENERAL LABORATORY TECHNIQUE.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoological Laboratory,  
University of Michigan, Ann Arbor, Mich.

**Sealing Mixtures for Bottles and Preparation Jars.** In a recent number of the *Pharmaceutical Era* (Vol. XXX, p. 528, 1903), the following formulæ for "bottle capping" mixtures are given. It is probable that they will prove useful in general laboratory practice:

I. Put a weighed amount of dry glue or gelatin in water and let it stand over night. In the morning drain and press off the superfluous water, and then dissolve the swollen mass by heating in a water bath. Add one-half as much glycerine as there is liquid gelatin and for every ounce of gelatin add 1 ounce of tannic acid. Stir until the mass is entirely homogeneous. Mineral colors may be used in case it is desired to color the sealing mixture. The mixture should be tested on glass before using. If, when dry, it is too hard or brittle add a little more glycerine, if too soft, add more glue and tannin, preserving the proportion between them indicated above.

II. Shellac,	-	-	-	-	-	-	3 ounces
Venice turpentine,	-	-	-	-	-	-	1½ ounces
Boric acid,	-	-	-	-	-	-	72 grains
Powdered talcum,	-	-	-	-	-	-	3 ounces
Ether,	-	-	-	-	-	-	6 fl. drams
Alcohol,	-	-	-	-	-	-	12½ fl. drams

Dissolve shellac, turpentine and boric acid in the mixed alcohol and ether. Color with some spirit soluble dye, and add the talcum. During use the mixture must be frequently agitated.

III. Collodion varnish,							
Pyroxylin,	-	-	-	-	-	-	1 ounce
Ether,	-	-	-	-	-	-	6 ounces
Alcohol	-	-	-	-	-	-	8 ounces

Dissolve and add camphor, 2½ drams. Aniline dyes may be used for coloring. When used the cork and part of the neck of the bottle should be dipped in the fluid.

R. P.

**The Demonstration of Nucleated Red Blood Corpuscles in Animal Spleens.** Under this title Dr. E. T. Williams describes in *Amer. Med.*, Vol. VI, pp. 855-856, his technique for fixing and staining erythroblasts. He finds it necessary in the first instance to have absolutely fresh material. He does not use spleens which have been kept more than six hours after death. The spleen of the hog is a suitable object. "The first step is the preparation of the slide. Take a fresh spleen, wash it carefully, and cut from the outer edge with a clean, sharp knife or razor, a wedge-shaped piece, like a piece of pie, about two inches long. Take it by the broad end between the thumb and forefinger and draw the raw point across the middle of a clean glass slide, without pressing or

squeezing, and as lightly as you would draw a camel's hair brush to dust the surface of a lens. The object is to make a thin smear. It is a good plan to make a dozen smears, and dry them at room heat under cover." The smears are fixed in the following fluid, on which, in large measure, the success of the process apparently depends :

Sublimate,	-	-	-	-	-	.78 gr. (xvii gr.)
Sodium chloride,	-	-	-	-	-	.28 gr. ( iv gr.)
Distilled water,	-	-	-	-	-	30 c. c. ( i oz.)
Dissolve and filter.						

"One minute's application of this solution makes a perfect fixative for spleen smears, without producing the slightest distortion of the red blood corpuscles, or the least impairment of their staining properties. They are then stained half an hour in watery solution of alum hæmatoxylin, washed, and counterstained from two to three minutes in a 3 per cent. watery eosin. These directions if carefully followed will be found to give perfect results." The preparations so made should be dried and examined without a cover-glass. With the immersion lens a low power ocular should be used till a corpuscle containing a nucleus is found. It may then be studied with a high power ocular.

R. P.

#### Technique for Dense Connective Tissue.

Dr. E. Retterer gives an account in a recent number of the *Journal de l'Anatomie* (Ann. 39, pp. 196, 1903) of the technique which he has developed in his work on the histology of the skin. It is of course well known that tissues containing a large amount of dense connective tissue are very troublesome objects for the microtomet. Retterer says that the things to be avoided in dealing with such tissues are too long a stay of the object in the alcohols, and too high a temperature at the time when the tissue is transferred to paraffin. His procedure in detail is as follows : Pieces of skin which have been fixed in either Flemming's, Zenker's, or Branca's fluid and have been washed are dehydrated in 90 per cent. alcohol (1 hour ca.) and absolute alcohol ( $\frac{1}{2}$  hour ca.). The tissue is next passed to xylol (20 minutes), followed by a mixture of xylol and 36° paraffin (30 minutes at 20°). It is then left for a quarter of an hour in 36° paraffin maintained at a temperature of 40°, an aspirator aiding in the infiltration. The tissue is then embedded in 54° paraffin in the following way : The paraffin in the embedding dish is allowed to set all around the edges and bottom of the dish, while a portion in the center is kept liquid. The tissue is placed in this melted portion and the whole allowed to cool, which it will do in about ten minutes. The tissue may be left in the block for some time before cutting, without injury to the histological elements. The author states that in all his experience he has never had a failure with the method.

R. P.

#### A Method of Making Frozen Sections of the Head.

In the *Journal of Anatomy and Physiology* (Vol. 37, p. 106, 1903) Prof. Symington describes a method which he has used with marked success in making macroscopic sections of a frozen human head. The entire body was hardened by the injection of a strong solution of formalin. The neck was divided opposite the fifth cervical vertebra and the head again injected with a solution of gum,

through the carotids. The head was put into a wooden box just large enough to hold it and the box was filled with gum solution. The closed box was kept in the freezing mixture until well frozen. The box was then fixed in a frame and sawn across along with its contents. The sections so obtained show extremely little displacement or distortion of the parts of the brain.

R. P.

**A Method of Preparing the Membranous Labyrinth.** Dr. A. A. Gray describes in the *Jour. Anat. and Physiol.*, N. S., Vol. 17,

Part IV, a method of making demonstration specimens of the membranous labyrinth of the human ear. Since it is a much neater method than any before described and will be found applicable in the case of small mammals, an account of it is given here for the benefit of JOURNAL readers. The principle of Dr. Gray's method is to embed the whole pyramid of the temporal bone so thoroughly that no acid can affect the soft parts, then to decalcify and *disintegrate* the bone so completely that no force whatever is required to remove the destroyed tissue surrounding the labyrinth, and finally to remove the embedding material in such a way that the membranous labyrinth is left uninjured.

The pyramid of the human temporal bone is removed from the base of the skull in the post-mortem room. The superfluous bone is removed with a saw; the stapes is carefully extracted from the oval window and a small hole is filled in the superior semicircular canal. The structure is then immersed in 90 per cent. alcohol for at least a fortnight, the alcohol being frequently changed. It is then transferred to absolute alcohol, where it remains at least a fortnight, this alcohol also being frequently changed. During this period it must be kept in a glass stoppered jar. From absolute alcohol the bone is removed quickly to xylol, where it again remains at least a fortnight, the xylol being frequently changed. If a vacuum be made in the jar above the liquid at intervals, diffusion occurs more rapidly and completely. From the xylol the bone is removed to melted paraffin of a melting point of 52° C. or 54° C. The paraffin must be changed two or three times during the fortnight which must be allowed for the embedding. This is important to the success of the method; the paraffin must permeate the soft parts far more completely than is necessary in the case of embedding for microscopic sectioning. A vacuum may be made above the paraffin, which will sometimes solidify during the process; this, however, does not appear to affect the ultimate result. Of course the temperature of the paraffin bath must not be allowed to rise above 55 or 56° C. After the bone has been in the paraffin bath for a period ranging from two to three weeks, the paraffin is cooled as quickly as possible and the bone cut out from the block. The superfluous paraffin is then carefully scraped off the bone and decalcification is proceeded with. For this purpose neither nitric nor hydrochloric acid by itself is suitable. They should be mixed. The best solution consists of 2 parts of nitric acid to 3 parts pure hydrochloric acid, and 6 to 18 parts water; the nitric acid should be mixed with the water first, and the hydrochloric acid added. The bone is put into a large quantity of this mixture and suspended near the top by fine twine. The mixture should be frequently changed, and in about three weeks or a month decalcification and disintegration will be complete. It will be found

that while the cancellous portions of the bone are still firm, being supported by the paraffin, the dense bone which surrounds the labyrinth will have become quite pulpy; indeed, the labyrinth should lie almost loose in the pulp; if this is not so, the preparation should be put back into the acid and left longer, and the solution should be made stronger by adding a little more hydrochloric acid. After decalcification the mass should be thoroughly washed for twenty-four hours in gently running water, care being taken that it does not get roughly handled during the process. Some of the cancellous portions of the bone may now be picked very carefully away with the sharp point of a knife, and then the mass is carefully removed to absolute alcohol, where it remains for about ten days, the alcohol being changed several times. From the alcohol it is transferred rapidly to xylol, which slowly dissolves out the paraffin and leaves the membranous labyrinth transparent. Surrounding portions of cancellous tissue may be slightly adherent, but they can be separated by fine sharp scissors as the structure lies in the xylol. If the dehydration has been thorough, extremely delicate structures do not collapse or shrink when the paraffin is removed. The specimen is preserved in xylol in the glass jar when the paraffin has been melted out. The courses of the various portions of the nerves stand out very plainly, and may be shown still more clearly if the specimen has previously been stained and fixed with osmic acid. Very pretty specimens may also be made by injecting the endolymph spaces (before hardening) with carmine gelatine, the injection being done through the aqueductus vestibuli. The blood vessels may be injected through the internal auditory artery, also of course before hardening. R. P.

**A Simple Apparatus and Method for** In the *Arch. f. Schiff- und Tropen-Hygiene* (Bd. VII, Heft 9, pp. 434-436, 1903), Dr. Markl describes a simple apparatus which he has used with much success in making chemical analyses of water. While his use of it has been for hygienic purposes, it seems evident from the account given that the apparatus will prove very useful for biologists who wish, in the course of ecological or experimental work, to make chemical analyses of water. The apparatus is made by the firm of Burroughs Wellcome & Co., in London. It consists of several glass cylinders, with graduations to 70 ccm., a shaking flask with ground glass stopper, a flask for boiling the water, an alcohol lamp with tripod foot, a nickel dish, several glass rods, and a number of accurately measured reagents in tablet form together with small sealed glass spheres containing Nessler's reagent. The tests are made in the following way: After the color, degree of turbidity and odor of the water have been determined a sample is boiled away in the nickel pan in order to estimate the content of dry substance. The hardness of the water is determined by adding to 70 ccm. of the water soap tablets till the foam formed by vigorous shaking persists. Each tablet indicates four degrees of hardness on the scale used. The chlorine is titrated out in a glass cylinder with silver nitrate pastilles, with the addition of a potassium bichromate tablet as an indicator. Each pastille indicates 2 mg. of chlorine. The organic matter is determined by means of tablets of potassium permanganate in hot solution. Each tablet indicates 1/10 mg. of absorbed oxygen. The presence of ammonia



is determined by the Nessler's reagent. Tablets are provided for the determination of nitric acid and nitrates, lead, zinc, iron, and copper. The author gives comparative tables of the results of the analysis of various samples of water by this method, and by the elaborate quantitative chemical methods, and shows that the method here described gives very satisfactory results.

R. P.

#### An Adjustable Anatomical Table.

In the *University of Pennsylvania Medical Bulletin* for June, 1903, Prof. G. G.

Davis describes a table which has proved very useful for human anatomical



FIG. 1.

demonstrations. It consists of a frame supporting a top, which is capable of being tilted, being hinged near the middle. The top is made of paraffined oak,

6.5 feet long by 2 feet wide. Across the under side, near the ends, are fastened two cleats to prevent warping. The frame on which the top rests is 39 inches long and as wide as the top. It is supported by four rollers 3.5 inches in diameter. The top is 3 feet above the floor. It is fastened to the frame by two stout hinges, placed a little beyond the middle, so that when it is raised to an upright position it clears the floor. Two semi-circles of iron are fastened to the sides, as shown in Fig. 1. They pass through clamps fastened on the sides of the frame. A thumb-screw at this point fastens the top in any desired position. Around the under edge of the top are twelve stout hooks, four on each side and four across the top. In using the table, the body having been placed on it is secured, first, by a Barton bandage applied to the head and fastened to the hooks at the top. Additional security can be given by bandages passing across the pelvis or other parts of the body and fastened to the hooks on the sides. The body having been secured in this manner, the top is prevented from tilting by a catch locking the upper end of the top to the frame. If it is desired to incline the top, the thumb screws on the sides are loosened, the catch at the top released, and pressure on the projecting end of the table causes the body to assume any degree of inclination desired. When the desired position is reached the thumb screws on the sides are turned and clamp the top in that position. The table with the inclined body can then be wheeled or turned as desired. It will be found of service in demonstrating the contents of the chest and abdomen, and is particularly useful in making anatomical sketches in which it is desirable to have the parts in a more or less upright position. Even when the body is in an absolutely vertical position the stability is perfectly satisfactory. Any good carpenter can make it.

R. P.

#### Method of Studying Living Bone Marrow.

A. Wolff describes in the *Deutsch. med. Wochenschr.* Bd. 10, S. 165, 1903, a method which he has found useful in studying living bone marrow. Long, tubular bones (e. g., the femur) of living animals are pierced under aseptic conditions. A small portion of the marrow is removed and mounted in an indifferent fluid. The amphiphile myelocytes may be seen to perform lively amœboid movements.

R. P.

#### A Method of Staining Sections Quickly with Picro-Carmine.

The following method of hastening the staining of sections with picro-carmine has been advocated by Freemann (*Proc. Physiol. Soc.*, 1903, pp. xxx and xxxi). The picro-carmine solutions used were Bourne's and Hoyer's.

1. To 1 volume of Bourne's picro-carmine 9 volumes of 0.2 per cent. acetic acid are added, the mixture is filtered, preferably after boiling. The sections are placed in the dilute picro-carmine, which is then heated quickly just to the boiling point, and allowed to cool. As the fluid cools, the sections stain; they are at their best in 3 to 4 minutes. Water may be used instead of dilute acetic acid, but the effect is not so good; the addition of acetic acid to picro-carmine in certain conditions has been recommended by Weigert.

2. To 1 volume of Hoyer's picro-carmine (solution made as directed by Hoyer) 19 volumes of water (best distilled) are added. The sections are treated

as above, but the staining is slower, taking 10 to 15 minutes instead of 3 to 4. For thick sections 4 volumes of water only should be added to 1 volume of picro-carmin, and the sections may be boiled in the mixture for 2 to 3 minutes.

The stain is stated to be chiefly that of carmine, but picric acid can be added to the alcohol through which the sections are passed after leaving the carmine. The method has been especially used on the central nervous system, and after the following hardening agents: Müller's fluid, potassium bichromate, Weigert's chrome-alum mixture and formalin. Sections hardened in chrome-alum, in which the medullated nerve fibres have been stained (Heller-Robertson method), can also be stained with carmine by the methods given above.

R. P.

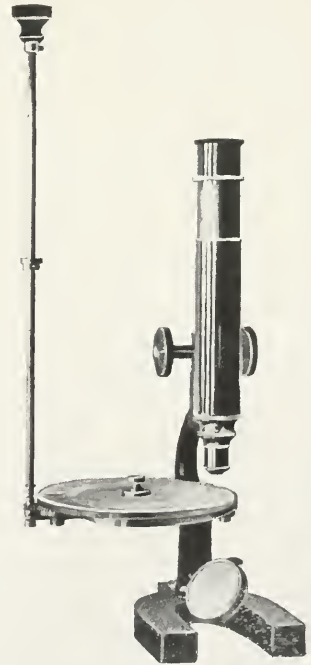
## NEWS AND NOTES.

THE following interesting information concerning a special feature of the government exhibit at St. Louis has been received from B. J. Howard, of the Bureau of Chemistry, United States Department of Agriculture:

"I am sending a print showing a new devise which we have invented in the form of an exhibition microscope to be used in connection with our exhibit at the St. Louis Exposition this summer. It consists of substituting a specially devised stage for the one furnished on the Bausch & Lomb stand, Style A. The new stage carries three cog-wheels so arranged that for each half revolution of the small pinion the large one is driven forward  $\frac{1}{20}$  revolution. The large wheel carries a circular plate-glass disk of 5 inches diameter attached to it by means of a thumb-screw passing through a hole in the center of the disk. The specimens are carefully mounted so that their centers are all an equal distance from the center of the disk, and covered with  $\frac{1}{2}$ -inch cover-glasses. In this way each disk will carry 20 specimens.

"On the shaft carrying the hard rubber button by which the small pinion is turned, is an intermittent gear arrangement (only part of which is seen in the print), which drives a dial with figures or names upon it, indicating each of the specimens as it comes into view under the objective.

"The whole instrument, with the exception of the ocular and hard rubber button, is enclosed in a glass case, the focusing being accomplished by means of raising or lowering the ocular—a set-screw preventing its being totally removed from the draw-tube."



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WITH this issue, completing the sixth volume of the JOURNAL OF APPLIED MICROSCOPY AND LABORATORY METHODS, its publication is closed.

On behalf of the publishers and readers of the JOURNAL, we wish to extend our appreciation to the many contributors and to the department editors who have, continuously and without remuneration other than the knowledge that they were working for the benefit of their co-laborers, supplied the material which has appeared in our pages.

The editor wishes to extend to the Bausch & Lomb Optical Company, and to Mr. Henry Lomb, through whose generosity the publication has been made possible, his appreciation of their liberality in this connection and of their interest in the advancement of science.

The publication was begun with the belief, based on extended observation, that as there was no publication in the English language devoted to laboratory methods and apparatus, those engaged professionally in laboratory work in colleges, universities, high schools and in the industries, as well as individual workers, would welcome such a publication especially if offered at a moderate price. Experience has shown that the leaders in this work have lent their support to the JOURNAL and have done all they could do to interest others, but the great mass of science teachers, those who are farthest from the great centers of investigation and growth, those who really need and could make daily use of the JOURNAL, and for whom it was primarily intended, have not evidenced as much interest as we could wish.

However, we believe that the JOURNAL has served a useful purpose, and that the complete volumes on file in the various libraries throughout the world will continue to be useful. That every special line of work is helped by the stimulus of a live periodical is evident, and we trust that, should others begin the publication of a journal for American Microscopy, it will receive the hearty support of all workers.

## QUESTION BOX.

Inquiries will be printed in this department from any inquirer.  
The replies will appear as received.

No. 35.—Kindly give the practical and also the theoretical formula for computing the value (focal length) of the supplementary lens for any length of camera extension, and any combination of lenses, eye-piece or tube length as per the Misses Foote and Strobell method of focusing for photo-micrography, as described in the April, 1902, and December, 1902, numbers of the JOURNAL, assuming the eye of operator to be normal and on the bright side of thirty years of age.—D. A. HALING, 39 Cheshire St., Cleveland, O.



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